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(54) Title: ASTHMA ASSOCIATED FACTORS AS TARGETS FOR TREATING ATOPIC ALLERGIES INCLUDING ASTHMA AND RELATED DISORDERS (57) Abstract A new gene in the calcium-activated chloride channel family has been discovered that is induced by IL-9, thereby providing a therapeutic target in IL-9 mediated development of atopic allergy, asthma-related disorders and cystic fibrosis. A method for the identification and use of small molecule inhibitors of this gene and its products to treat these disorders has also been discovered. The invention also includes a method for diagnosing susceptibility to, and assessing treatment of atopic allergy, asthma-related disorders by measuring the level of gene expression in biologic samples using antibody specific for this protein.		

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**ASTHMA ASSOCIATED FACTORS AS TARGETS FOR
TREATING ATOPIC ALLERGIES INCLUDING
ASTHMA AND RELATED DISORDERS**

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/076,815 which was filed March 3, 1998 and which is herein incorporated by reference in its entirety. This invention is also related to the subject matter of U.S. Patent
5 Application Serial Nos. 08/697,419; 08/697,360; 08/697,473; 08/697,472; 08/697,471; 08/702,105; 08/702,110; 08/702,168; and 08/697,440, all of which were filed on August 23, 1996 and are incorporated herein by reference. This application is also related to U.S. Patent Application Serial No. 08/980,872 which was filed December 1, 1997 and which is incorporated herein by reference.

10 FIELD OF THE INVENTION

This invention relates to modulating activities associated with the IL-9 pathway for the treatment of atopic allergies and related disorders such as asthma.

BACKGROUND OF THE INVENTION

Inflammation is a complex process in which the body's defense system combats
15 foreign entities. While the battle against foreign entities may be necessary for the body's survival, some defense systems respond to foreign entities, even innocuous ones, as dangerous and thereby damage surrounding tissue in the ensuing battle.

Atopic allergy is an ecogenetic disorder, where genetic background dictates the response to environmental stimuli. The disorder is generally characterized by an
20 increased ability of lymphocytes to produce IgE antibodies in response to ubiquitous antigens. Activation of the immune system by these antigens leads to allergic inflammation and may occur after ingestion, penetration through the skin or after inhalation. When this immune activation occurs and is accompanied by pulmonary

inflammation and bronchial hyperresponsiveness, this disorder is broadly characterized as asthma. Certain cells are important in this inflammatory reaction and they include T cells and antigen-presenting cells, B cells that produce IgE, basophils that bind IgE and eosinophils. These inflammatory cells accumulate at the site of allergic inflammation and the toxic products they release contribute to tissue destruction related to these disorders.

While asthma is generally defined as an inflammatory disorder of the airways, clinical symptoms arise from intermittent air flow obstruction. It is a chronic, disabling disorder that appears to be increasing in prevalence and severity (Gergen *et al.*, 1992). It is estimated that 30-40% of the population suffer with atopic allergy and 15% of children and 5% of adults in the population suffer from asthma (Gergen *et al.*, 1992). Thus, an enormous burden is placed on health-care resources.

Interestingly, while most individuals experience similar environmental exposures, only certain individuals develop atopic allergy and asthma. This hypersensitivity to environmental allergens known as "atopy" is often indicated by elevated serum IgE levels or abnormally intense skin test response to allergens in atopic individuals as compared to nonatopics (Marsh *et al.*, 1982). Strong evidence for a close relationship between atopic allergy and asthma is derived from the fact that most asthmatics have clinical and serologic evidence of atopy (Clifford *et al.*, 1987; Gergen, 1991; Burrows *et al.*, 1992; Johannson *et al.*, 1972; Sears *et al.*, 1991; Halonen *et al.*, 1992). In particular, younger asthmatics have a high incidence of atopy (Marsh *et al.*, 1982). In addition, immunologic factors associated with an increase in total serum IgE levels are very closely related to impaired pulmonary function (Burrows *et al.*, 1989).

Both the diagnosis and treatment of these disorders are problematic (Gergen *et al.*, 1992). The assessment of inflamed lung tissue is often difficult and frequently the source of the inflammation cannot be determined. It is now generally accepted that failure to control pulmonary inflammation leads to significant loss of lung function over time.

Current treatments suffer their own set of disadvantages. The main therapeutic agents, β agonists, reduce the symptoms thereby transiently improving pulmonary function, but do not affect the underlying inflammation so that lung tissue remains in jeopardy. In addition, constant use of β agonists results in desensitization which reduces

their efficacy and safety (Molinoff *et al.*, 1995). The agents that can diminish the underlying inflammation, such as anti-inflammatory steroids, have their own list of disadvantages that range from immunosuppression to bone loss (Molinoff *et al.*, 1995).

Because of the problems associated with conventional therapies, alternative treatment strategies have been evaluated. Glycophorin A (Chu *et al.*, 1992), cyclosporin (Alexander *et al.*, 1992; Morely, 1992) and a nonapeptide fragment of interleukin 2 (IL-2) (Zavvalov *et al.*, 1992) all inhibit potentially critical immune functions associated with homeostasis. What is needed in the art is a treatment for asthma that addresses the underlying pathogenesis. Moreover, these therapies must address the episodic nature of the disorder and the close association with allergy and intervene at a point downstream from critical immune functions.

In the related patent applications mentioned above, applicants have demonstrated that interleukin 9 (IL-9), its receptor and activities effected by IL-9 are the appropriate targets for therapeutic intervention in atopic allergy, asthma and related disorders.

Mediator release from mast cells by allergen has long been considered a critical initiating event in allergy. IL-9 was originally identified as a mast cell growth factor and it has been demonstrated that IL-9 up-regulates the expression of mast cell proteases including MCP-1, MCP-2, MCP-4 (Eklund *et al.*, 1993) and granzyme B (Louahed *et al.*, 1995). Thus, IL-9 appears to serve a role in the proliferation and differentiation of mast cells. Moreover, IL-9 up-regulates the expression of the alpha chain of the high affinity IgE receptor (Dugas *et al.*, 1993). Elevated IgE levels are considered to be a hallmark of atopic allergy and a risk factor for asthma. Furthermore, both *in vitro* and *in vivo* studies have shown IL-9 to potentiate the release of IgE from primed B cells (Petit-Frere *et al.*, 1993).

There is substantial support for the role of IL-9 gene in asthma. First, linkage homology between humans and mice suggests that the same gene is responsible for producing biologic variability in response to antigen in both species. Second, differences in expression of the murine IL-9 candidate gene are associated with biologic variability in bronchial responsiveness. In particular, reduced expression of IL-9 is associated with a lower baseline bronchial response in B6 mice. Third, recent evidence for linkage disequilibrium in data from humans suggests IL-9 may be associated with atopy and

bronchial hyperresponsiveness consistent with a role for this gene in both species (Doull *et al.*, 1992). Moreover, a genetic alteration in the human gene appears to be associated with loss of cytokine function and lower IgE levels. Fourth, the pleiotropic functions of this cytokine and its receptor in the allergic immune response strongly support a role for the IL-9 pathway in the complex pathogenesis of asthma. Fifth, in humans, biologic variability in the IL-9 receptor also appears to be associated with atopic allergy and asthma. Finally, despite the inherited loss of IL-9 receptor function, these individuals appear to be otherwise healthy. Thus, nature has demonstrated in atopic individuals that the therapeutic down-regulation of IL-9 and IL-9 receptor genes or genes activated by IL-9 and its receptor is likely to be safe.

While the role of the IL-9 gene, its receptor and their functions in atopic allergy, asthma and related disorders has been elucidated, a specific need in the art exists for elucidation of the role of genes which are regulated by IL-9 in the etiology of these disorders. Furthermore, most significantly, based on this knowledge, there is a need for the identification of agents that are capable of regulating the activity of these genes or their gene products for treating these disorders.

Cystic fibrosis is yet another disease which effects the lung and is associated with thick secretions resulting in airway obstruction and subsequent colonization and infection by inhaled pathogenic microorganisms (Eng *et al.*, 1996). Cystic fibrosis airway epithelia exhibit a spectrum of ion transport properties that differ from normal, including not only defective cAMP-mediated chloride secretion, but also increased sodium absorption and increased calcium-mediated chloride secretion (Johnson *et al.*, 1995). The increase in calcium-mediated chloride secretion is presumably an attempt to compensate for the overall decrease in chloride secretion due to the defect in cAMP-mediated chloride secretion. It does not adequately compensate for this defect, however, because normal chloride gradients are not maintained. Thus, potential therapeutic remedies for cystic fibrosis rely on mechanisms which increase chloride secretion in airway epithelial cells to compensate for defective cAMP-mediated chloride secretion. Such mechanisms are capable of restoring the cellular chloride gradient thereby alleviating sodium hyperabsorption associated with decreased chloride secretion. A specific need in the art therefore exists for identification of agents capable of enhancing

calcium-dependent chloride secretion in cystic fibrosis airway epithelial cells.

SUMMARY OF THE INVENTION

The present invention includes new genes from the calcium activated chloride channel gene family designated ICACC (IL-9 Induced Calcium Activated Chloride Channel) , particularly the mouse (SEQ ID NO:1) and human (SEQ ID NO:3 and SEQ ID NO:5) ICACC genes. The ICACC-1 genes are selectively up-regulated by IL-9 and therefore part of the IL-9 signaling pathway. The present invention also includes the protein products of the ICACC genes, particularly, the mouse (SEQ ID NO:2) and human (SEQ ID NO:4 and NO:6) ICACC genes.

10 The inventors have satisfied the need for diagnosis and treatment of atopic allergy, asthma and related disorders by demonstrating the role of ICACC-1 in the pathogenesis of these disorders. Therapies for these disorders are derived from the down-regulation of ICACC-1 as a member of the IL-9 pathway.

The identification of ICACC-1 has led to the discovery of compounds that are capable of down-regulating its activity. Activity is defined here as any alteration in either chloride channel function or expression of ICACC-1. Molecules that down-regulate ICACC-1 are therefore part of the invention. Down-regulation is defined here as a decrease in activation, function or synthesis of ICACC-1, its ligands or activators. It is further defined to include an increase in the degradation of ICACC-1 gene, its protein product, ligands or activators. Down-regulation is therefore achieved in a number of ways. For example, administration of molecules that can destabilize the binding of ICACC-1 with its ligands. Such molecules encompass polypeptide products, including those encoded by the DNA sequences of the ICACC-1 gene or DNA sequences containing various mutations. These mutations may be point mutations, insertions, deletions or spliced variants of the ICACC-1 gene. This invention also includes truncated polypeptides encoded by the DNA molecules described above. These polypeptides being capable of interfering with interaction of ICACC-1 with its ligand and other proteins.

A further embodiment of this invention includes the down-regulation of ICACC-1 function by altering expression of the ICACC-1 gene, the use of antisense gene therapy

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being an example. Down-regulation of ICACC-1 expression is accomplished by administering an effective amount of antisense oligonucleotides. These antisense molecules can be fashioned from the DNA sequence of the ICACC-1 gene or sequences containing various mutations, deletions, insertions or spliced variants. Another
5 embodiment of this invention relates to the use of isolated RNA or DNA sequences derived from the ICACC-1 gene. These sequences containing various mutations such as point mutations, insertions, deletions or spliced variant mutations of ICACC-1 gene and can be useful in gene therapy.

Molecules that increase the degradation of the ICACC-1 protein may also be used to
10 down-regulate its functions and are within the scope of the invention. Phosphorylation of ICACC-1 may alter protein stability, therefore kinase inhibitors may be used to down-regulate its function. Down-regulation of ICACC-1 may also be accomplished by the use of polyclonal or monoclonal antibodies or fragments thereof directed against the ICACC-1 protein. Such molecules are within the claimed invention. This invention
15 further includes small molecules with the three-dimensional structure necessary to bind with sufficient affinity to block ICACC-1 interactions with its ligands or block function of the chloride channel. ICACC-1 blockade resulting in deregulation of calcium and chloride flux and other processes of proinflammatory cells where it is expressed make these small molecules useful as therapeutic agents in treating inflammation associated
20 with atopic allergy, asthma and related disorders. In a further embodiment, aminosterol compounds are assessed for their ability to block ICACC-1 induction by IL-9 or antigen as a means of determining their usefulness in treating atopic allergies and related disorders.

The agents discussed above represent various effective therapeutic compounds in
25 treating atopic allergies, asthma and other related disorders. Applicants have thus provided antagonists and methods of identifying antagonists that are capable of down-regulating ICACC-1. Applicants also provide methods for down-regulating the activity of ICACC-1 by administering truncated protein products, chloride channel blockers, aminosterols and the like.

30 Applicants also provide a method for the diagnosis of susceptibility to atopic allergy, asthma and related disorders by describing a method for assaying the induction of

ICACC-1, its functions or downstream activities. In a further embodiment, Applicants provide methods to monitor the effects of ICACC-1 down-regulation as a means to follow the treatment of atopic allergy and asthma. Applicants also provide a method for diagnosing autoimmune type diseases such as inflammatory bowel disease (IBD) where suppression of TH2-associated responses (such as the biologic responses associated with IL-9) are a common molecular feature. The constitutive expression of ICACC-1 in the small intestine and colon suggest that this is a useful marker for monitoring treatment of TH1 associated disease states such as IBD, where down regulated expression of ICACC-1 will be associated with the disease.

- 10 In a further embodiment, Applicants identify a disease state, which can be treated through the up-regulation of ICACC-1. Applicants provide a method for treating the defect in cAMP-mediated chloride secretion in cystic fibrosis airway epithelia by further increasing calcium-dependent chloride secretion through up-regulation of ICACC-1. This up-regulation of ICACC-1 resulting in increased chloride secretion and thus
- 15 restoration of the cellular chloride gradient resulting in normal airway epithelial cell function. Applicants provide a method for treating inflammatory bowel disease (IBD) with local delivery of ICACC-1 via gene therapy or up regulation of ICACC-1 to enhance TH2-associated responses for suppressing the TH1-associated IBD autoimmune disease.
- 20 The accompanying figures, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the invention and together with the description, serve to explain the principle of the invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a schematic diagram of the suppressive PCR cDNA subtraction technique.

Figure 2 shows the nucleotide (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequence of the murine ICACC-1 cDNA.

Figure 3 shows an alignment of the murine ICACC-1 protein with a bovine calcium activated chloride channel.

30 Figure 4A shows the nucleotide (SEQ ID NO:3) and amino acid (SEQ ID NO:4)

sequence of the human ICACC-2 cDNA.

Figure 4B shows the nucleotide (SEQ ID NO:5) and amino acid (SEQ ID NO:6) sequence of the human ICACC-1 cDNA.

Figure 5 shows an alignment of the murine ICACC-1 protein with the human
5 ICACC-1 and ICACC-2 protein.

Figure 6 shows ICACC-1 expression in the lung of normal mice (FVB) compared to transgenic mice overexpressing the IL-9 gene (Tg5).

Figure 7 shows the expression of ICACC-1 in the lungs of DBA and C57B6 mice.

Figure 8 shows the expression of ICACC-1 in the lung of the C57B6 mouse with and
10 without intratracheal administration of IL-9.

Figure 9 shows the expression of ICACC-1 in tissues from normal (Balb/C) and IL-9 overexpressing (Tg5) mice.

Figure 10 shows *Aspergillus fumigatus*-antigen induced BHR and eosinophilia in Balb/C mice.

Figure 11 shows the tissue distribution of ICACC-1 in naïve and antigen exposed
15 Balb/C mice.

Figure 12 shows the suppression of BHR and lung eosinophilia by anti-IL9 in mice exposed to *Aspergillus fumigatus*.

Figure 13 shows suppression of ICACC-1 in antigen exposed animals treated with
20 anti-IL9.

Figure 14 shows ICACC-1 induction by IL-9 in human primary lung epithelial cells (NHBE).

Figure 15 shows ICACC-1 induction by IL-9 in human primary lung cultures.

Figure 16 shows antisera generated against ICACC-1 peptides is able to recognize
25 native ICACC-1

Figure 17 shows IL-9 induces eotaxin production from epithelial cells in primary lung cultures

Figure 18 shows suppression of IL-9 induced eotaxin by chloride channel blockers

DETAILED DESCRIPTION OF THE INVENTION

The inventors have resolved a crucial need in the art by elucidating critical genes in the IL-9 pathway and compositions affecting that pathway which may be used in the diagnosis, prevention or treatment of atopic allergy including asthma and related disorders. Asthma encompasses inflammatory disorders of the airways with reversible airflow obstruction. Atopic allergy refers to atopy and related disorders including asthma, bronchial hyperresponsiveness, rhinitis, urticaria, allergic inflammatory disorders of the bowel and various forms of eczema. Atopy is a hypersensitivity to environmental allergens expressed as the elevation of serum total IgE or abnormal skin test responses to allergens as compared to controls. Bronchial hyperresponsiveness is a heightened broncho constrictor response to a variety of stimuli.

A. ICACC Proteins

The present invention provides isolated ICACC protein, allelic variants of the protein, and conservative amino acid substitutions of the protein. As used herein, the ICACC protein or polypeptide includes a protein that has the murine amino acid sequence of SEQ ID NO: 2 or the human amino acid sequence depicted in SEQ ID No.4 or SEQ ID No.6. The invention includes naturally occurring allelic variants and proteins that have a slightly different amino acid sequence than that specifically recited above. Allelic variants, though possessing a slightly different amino acid sequence than those recited above, will still have the same or similar biological functions associated with the ICACC protein.

As used herein, the family of proteins related to the ICACC protein refer to proteins that have been isolated from organisms in addition to humans or mice. The methods used to identify and isolate other members of the family of proteins related to the human and/or murine ICACC proteins are described below.

The proteins of the present invention are preferably in isolated form. As used herein, a protein is said to be isolated when physical, mechanical or chemical methods are employed to remove the protein from cellular constituents that are normally associated with the protein. A partially isolated protein, as used herein, includes ICACC proteins isolated in membrane fragments, including cellular membrane fragments containing a

recombinantly expressed ICACC protein. A skilled artisan can readily employ standard purification methods to obtain an isolated protein.

The proteins of the present invention further include conservative variants of the proteins herein described. As used herein, a conservative variant refers to alterations in
5 the amino acid sequence that do not adversely affect the biological functions of the protein. A substitution, insertion or deletion is said to adversely affect the protein when the altered sequence prevents or disrupts a biological function associated with the protein. For example, the overall charge, structure or hydrophobic/hydrophilic properties of the protein may be altered without adversely affecting a biological activity.

10 Accordingly, the amino acid sequence can be altered, for example to render the peptide more hydrophobic or hydrophilic, without adversely affecting the biological activities of the protein.

Ordinarily, the allelic variants, the conservative substitution variants, and the members of the protein family, will have an amino acid sequence having at least about
15 55%, at least about 75% amino acid sequence identity with the murine sequence set forth in SEQ ID No.2 or the human sequences of SEQ ID NO: 4 or SEQ ID No.6, more preferably at least 80%, even more preferably at least 90%, and most preferably at least 95%. Identity or homology with respect to such sequences is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the
20 known peptides, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. N-terminal, C-terminal or internal extensions, deletions, or insertions into the peptide sequence shall not be construed as affecting homology.

25 Thus, the proteins of the present invention include molecules having the amino acid sequence disclosed in SEQ ID Nos. 2, 4 or 6; fragments thereof having a consecutive sequence of at least about 3, 4, 5, 6, 10, 15, 20, 25, 30, 35 or more amino acid residues of the ICACC protein; amino acid sequence variants of such sequence wherein an amino acid residue has been inserted N- or C-terminal to, or within, the disclosed sequence; and
30 amino acid sequence variants of the disclosed sequence, or their fragments as defined above, that have been substituted by another residue. Contemplated variants further

include those containing predetermined mutations by, *e.g.*, homologous recombination, site-directed or PCR mutagenesis, and the corresponding proteins of other animal species, including but not limited to rabbit, rat, porcine, bovine, ovine, equine and non-human primate species, and the alleles or other naturally occurring variants of the family
5 of proteins; and derivatives wherein the protein has been covalently modified by substitution, chemical, enzymatic, or other appropriate means with a moiety other than a naturally occurring amino acid (for example a detectable moiety such as an enzyme or radioisotope).

As described below, members of the family of proteins can be used: 1) to identify
10 agents which modulate at least one activity of the protein; 2) in methods of identifying binding partners for the protein; and 3) as an antigen to raise polyclonal or monoclonal antibodies.

B. Nucleic Acid Molecules

The murine ICACC-1 gene was identified by subtractive cDNA cloning experiments
15 that were performed in order to identify genes specifically induced by IL-9. A schematic diagram of the subtractive cDNA cloning method is provided in Figure 1. RNA derived from lungs of transgenic mice overexpressing the murine IL-9 transgene (Tg5) was used to isolate genes expressed in response to IL-9 as opposed to those which are not expressed in the parental strain (FVB). Figure 6 shows a Northern blot with RNA from a
20 lung of a Tg5 mouse (right lane) and a FVB mouse (left lane) demonstrating these findings. Expression of ICACC-1 was also observed in the lung of the DBA murine strain which has been shown to express elevated baseline IL-9 levels in their lungs (Figure 7). ICACC-1 expression was not observed in the lungs of the C57B6 strain where IL-9 expression is below the limits of detection (Figure 7) (Nicolaidis *et al.*,
25 1997). The direct effect of IL-9 on inducing ICACC-1 expression was demonstrated when IL-9 was instilled into the trachea of the C57B6 mouse. The results of this experiment demonstrated that ICACC-1 was expressed in the lungs of the IL-9 instilled mice but not in naive or vehicle treated mice (Figure 8), indicating that this gene is induced by IL-9. The results also show that ICACC-1 gene expression is induced in the
30 lung of antigen exposed mice which exhibit asthmatic-like features (BHR, lung

eosinophilia) (Figures 10 and 12). The antigen induced BHR and lung eosinophilia can be suppressed in mice by treatment with anti-IL9 (Figure 12), which also results in down regulation of ICACC-1 (Figure 13).

The murine ICACC-1 gene displayed significant homology (~50%) with a member
5 of the bovine calcium activated chloride channel family (Figure 3) (Cunningham *et al.*, 1995). The full length cDNA was cloned from a murine cDNA library (Figure 2). Several EST were identified which displayed partial homology to the murine ICACC-1. These EST were obtained from the IMAGE consortium (Lawrence Livermore National Laboratory) and sequenced. A full length cDNA sequence was isolated for human
10 ICACC-1 and 2 by library screening and 5'-and 3' RACE cloning (Clontech). Analysis of the encoded murine protein sequence identified several conserved motifs including multiple transmembrane domains and several phosphorylation and glycosylation sites.

Expression of murine ICACC-1 was undetectable using standard commercial tissue blots but elevated expression of ICACC-1 was observed in lung, lymph node, colon,
15 spleen, stomach, uterus and ovary derived from IL-9 transgenic mice (Figure 9). Interestingly, these tissues all contain various epithelial cell types, suggesting that this gene may be restricted to IL-9 responsive epithelial cells. This data is supported by the finding that ICACC-1 gene expression is induced in antigen exposed mice and this induction can be suppressed by anti-IL9 treatment (Figures 10, 12, and 13).

20 To further understand which cell type is capable of expressing ICACC-1, Applicants tested human lung epithelial cells for their responsiveness to IL-9 as determined by ICACC-1 induced gene expression. As shown in Figure 14, the human lung epithelial cell line designated NHBE (Clontech), expressed ICACC-1 mRNA when grown in the presence, but not in the absence of IL-9. When human primary lung cultures were grown
25 in the presence of recombinant IL-9, ICACC-1 expression was induced in contrast to cell cultures grown in medium alone (Figure 15).

The nucleic acid molecules of the present invention include nucleic acid molecules that encode the proteins having SEQ ID No.2, SEQ ID No.4, SEQ ID NO: 6 and the related proteins herein described, preferably in isolated form. As used herein, "nucleic
30 acid" is defined as RNA or DNA that encodes a protein or peptide as defined above, or is complementary to nucleic acid sequence encoding such peptides, or hybridizes to such

nucleic acid and remains stably bound to it under appropriate stringency conditions, or encodes a polypeptide sharing at least 55% sequence identity, 75 % sequence identity, preferably at least 80%, and more preferably at least 85%, with the peptide sequences. Specifically contemplated are genomic DNA, cDNA, mRNA and antisense molecules, as well as nucleic acids based on alternative backbones or including alternative bases whether derived from natural sources or synthesized. Such hybridizing or complementary nucleic acids, however, are defined further as being novel and unobvious over any prior art nucleic acid including that which encodes, hybridizes under appropriate stringency conditions, or is complementary to nucleic acid encoding a protein according to the present invention.

Homology or identity is determined by **BLAST** (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs **blastp**, **blastn**, **blastx**, **tblastn** and **tblastx** (Karlin , *et al.* Proc. Natl. Acad. Sci. USA 87: 2264-2268 (1990) and Altschul, S. F. J. Mol. Evol. 36: 290-300(1993), fully incorporated by reference) which are tailored for sequence similarity searching. The approach used by the **BLAST** program is to first consider similar segments between a query sequence and a database sequence, then to evaluate the statistical significance of all matches that are identified and finally to summarize only those matches which satisfy a preselected threshold of significance. For a discussion of basic issues in similarity searching of sequence databases, see Altschul *et al.* (Nature Genetics 6: 119-129 (1994)) which is fully incorporated by reference. The search parameters for **histogram**, **descriptions**, **alignments**, **expect** (i.e., the statistical significance threshold for reporting matches against database sequences), **cutoff**, **matrix** and **filter** are at the default settings. The default scoring matrix used by **blastp**, **blastx**, **tblastn**, and **tblastx** is the **BLOSUM62** matrix (Henikoff, *et al.* Proc. Natl. Acad. Sci. USA 89: 10915-10919 (1992), fully incorporated by reference). For **blastn**, the scoring matrix is set by the ratios of **M** (i.e., the reward score for a pair of matching residues) to **N** (i.e., the penalty score for mismatching residues), wherein the default values for **M** and **N** are 5 and -4, respectively.

"Stringent conditions" are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015M NaCl/0.0015M sodium titrate/0.1% SDS

at 50°C., or (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is use of 50% formamide, 5 x SSC (0.75M
5 NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C., with washes at 42°C. in 0.2 x SSC and 0.1% SDS. A skilled artisan can readily determine and vary the stringency conditions appropriately to obtain a clear and detectable hybridization signal.

10 As used herein, a nucleic acid molecule is said to be "isolated" when the nucleic acid molecule is substantially separated from contaminant nucleic acid encoding other polypeptides from the source of nucleic acid.

The present invention further provides fragments of the encoding nucleic acid molecule. As used herein, a fragment of an encoding nucleic acid molecule refers to a small portion of
15 the entire protein encoding sequence. The size of the fragment will be determined by the intended use. For example, if the fragment is chosen so as to encode an active portion of the protein, the fragment will need to be large enough to encode the functional region(s) of the protein or may encode regions of homology between the ICACC proteins in Figure 5. If the fragment is to be used as a nucleic acid probe or PCR primer, then the fragment length is
20 chosen so as to obtain a relatively small number of false positives during probing/priming.

Fragments of the encoding nucleic acid molecules of the present invention (i.e., synthetic oligonucleotides) that are used as probes or specific primers for the polymerase chain reaction (PCR), or to synthesize gene sequences encoding proteins of the invention can easily be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci, *et*
25 *al.*, (*J. Am. Chem. Soc.* 103:3185-3191, 1981) or using automated synthesis methods. In addition, larger DNA segments can readily be prepared by well known methods, such as synthesis of a group of oligonucleotides that define various modular segments of the gene, followed by ligation of oligonucleotides to build the complete modified gene.

The encoding nucleic acid molecules of the present invention may further be modified so
30 as to contain a detectable label for diagnostic and probe purposes. A variety of such labels are known in the art and can readily be employed with the encoding molecules herein

described. Suitable labels include, but are not limited to, biotin, radiolabeled nucleotides and the like. A skilled artisan can employ any of the art known labels to obtain a labeled encoding nucleic acid molecule.

Modifications to the primary structure itself by deletion, addition, or alteration of the amino acids incorporated into the protein sequence during translation can be made without destroying the activity of the protein. Such substitutions or other alterations result in proteins having an amino acid sequence encoded by a nucleic acid falling within the contemplated scope of the present invention.

C. Isolation of Other Related Nucleic Acid Molecules

As described above, the identification of the murine nucleic acid molecule having SEQ ID NO:1 and the human nucleic acid molecules having SEQ ID No.3 or SEQ ID No 5 allows a skilled artisan to isolate nucleic acid molecules that encode other members of the ICACC protein family in addition to the murine or human sequences herein described.

Essentially, a skilled artisan can readily use the amino acid sequence of SEQ ID NOS: 2, 4 or 6 to generate antibody probes to screen expression libraries prepared from appropriate cells. Typically, polyclonal antiserum from mammals such as rabbits immunized with the purified protein (as described below) or monoclonal antibodies can be used to probe a mammalian cDNA or genomic expression library, such as lambda gtl1 library, to obtain the appropriate coding sequence for other members of the protein family. The cloned cDNA sequence can be expressed as a fusion protein, expressed directly using its own control sequences, or expressed by constructions using control sequences appropriate to the particular host used for expression of the enzyme.

Alternatively, a portion of the coding sequence herein described can be synthesized and used as a probe to retrieve DNA encoding a member of the protein family from any mammalian organism. Oligomers containing approximately 18-20 nucleotides (encoding about a 6-7 amino acid stretch) are prepared and used to screen genomic DNA or cDNA libraries to obtain hybridization under stringent conditions or conditions of sufficient stringency to eliminate an undue level of false positives.

Additionally, pairs of oligonucleotide primers can be prepared for use in a polymerase chain reaction (PCR) to selectively clone an encoding nucleic acid molecule. A PCR

denature/anneal/extend cycle for using such PCR primers is well known in the art and can readily be adapted for use in isolating other encoding nucleic acid molecules.

D. rDNA molecules Containing a Nucleic Acid Molecule

The present invention further provides recombinant DNA molecules (rDNAs) that
5 contain a ICACC coding sequence. As used herein, a rDNA molecule is a DNA molecule that has been subjected to molecular manipulation *in situ*. Methods for generating rDNA molecules are well known in the art, for example, see Sambrook *et al.*, *Molecular Cloning* (1989). In the preferred rDNA molecules, a coding DNA sequence is operably linked to expression control sequences and/or vector sequences.

10 The choice of vector and/or expression control sequences to which one of the protein family encoding sequences of the present invention is operably linked depends directly, as is well known in the art, on the functional properties desired, e.g., protein expression, and the host cell to be transformed. A vector contemplated by the present invention is at least capable of directing the replication or insertion into the host chromosome, and preferably also
15 expression, of the structural gene included in the rDNA molecule.

Expression control elements that are used for regulating the expression of an operably linked protein encoding sequence are known in the art and include, but are not limited to, inducible promoters, constitutive promoters, secretion signals, and other regulatory elements. Preferably, the inducible promoter is readily controlled, such as being responsive to a nutrient
20 in the host cell's medium.

In one embodiment, the vector containing a coding nucleic acid molecule will include a prokaryotic replicon, i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extrachromosomally in a prokaryotic host cell, such as a bacterial host cell, transformed therewith. Such replicons are
25 well known in the art. In addition, vectors that include a prokaryotic replicon may also include a gene whose expression confers a detectable marker such as a drug resistance. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline.

Vectors that include a prokaryotic replicon can further include a prokaryotic or
30 bacteriophage promoter capable of directing the expression (transcription and translation) of

the coding gene sequences in a bacterial host cell, such as *E. coli*. A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322 and pBR329 available from Biorad Laboratories, (Richmond, CA), pPL and pKK223 available from Pharmacia, Piscataway, N.J.

Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can also be used to form a rDNA molecules that contains a coding sequence. Eukaryotic cell expression vectors are well known in the art and are available from several commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired DNA segment. Typical of such vectors are pSVL and pKSV-10 (Pharmacia), pBPV-1/pML2d (International Biotechnologies, Inc.), pTDT1 (ATCC, #31255), the vector pCDM8 described herein, and the like eukaryotic expression vectors.

Eukaryotic cell expression vectors used to construct the rDNA molecules of the present invention may further include a selectable marker that is effective in an eukaryotic cell, preferably a drug resistance selection marker. A preferred drug resistance marker is the gene whose expression results in neomycin resistance, i.e., the neomycin phosphotransferase (*neo*) gene. (Southern *et al.*, *J. Mol. Anal. Genet.* 1:327-341, 1982.) Alternatively, the selectable marker can be present on a separate plasmid, and the two vectors are introduced by co-transfection of the host cell, and selected by culturing in the appropriate drug for the selectable marker.

E. Host Cells Containing an Exogenously Supplied Coding Nucleic Acid Molecule

The present invention further provides host cells transformed with a nucleic acid molecule that encodes an ICACC protein, preferably an ICACC-1 protein, of the present invention. The host cell can be either prokaryotic or eukaryotic. Eukaryotic cells useful for expression of a protein of the invention are not limited, so long as the cell line is compatible with cell culture methods and compatible with the propagation of the expression vector and expression of the gene product. Preferred eukaryotic host cells include, but are not limited to,

yeast, insect and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human cell line. Preferred eukaryotic host cells include Chinese hamster ovary (CHO) cells available from the ATCC as CCL61, NIH Swiss mouse embryo cells NIH/3T3 available from the ATCC as CRL 1658, baby hamster kidney cells (BHK), and the
5 like eukaryotic tissue culture cell lines.

Any prokaryotic host can be used to express a rDNA molecule encoding a protein of the invention. The preferred prokaryotic host is *E. coli*.

Transformation of appropriate cell hosts with a rDNA molecule of the present invention is accomplished by well known methods that typically depend on the type of vector used and
10 host system employed. With regard to transformation of prokaryotic host cells, electroporation and salt treatment methods are typically employed, see, for example, Cohen *et al.*, *Proc. Natl. Acad. Sci. USA* 69:2110, 1972; and Maniatis *et al.*, Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982). With regard to transformation of vertebrate cells with vectors containing rDNAs,
15 electroporation, cationic lipid or salt treatment methods are typically employed, see, for example, Graham *et al.*, *Viol.* 52:456, 1973; Wigler *et al.*, *Proc. Natl. Acad. Sci. USA* 76:1373-76, 1979.

Successfully transformed cells, i.e., cells that contain a rDNA molecule of the present invention, can be identified by well known techniques including the selection for a selectable
20 marker. For example, cells resulting from the introduction of an rDNA of the present invention can be cloned to produce single colonies. Cells from those colonies can be harvested, lysed and their DNA content examined for the presence of the rDNA using a method such as that described by Southern, *J. Mol. Biol.* 98:503, 1975, or Berent *et al.*, *Biotech.* 3:208, 1985 or the proteins produced from the cell assayed via an immunological
25 method.

F. Production of Recombinant Proteins using a rDNA Molecule

The present invention further provides methods for producing an ICACC protein of the invention using nucleic acid molecules herein described. In general terms, the production of a recombinant form of a protein typically involves the following steps:

30 First, a nucleic acid molecule is obtained that encodes a protein of the invention, such as

the nucleic acid molecule depicted in SEQ ID NOS.1, 3 or 5 or the open reading frames of these molecules. If the encoding sequence is uninterrupted by introns, it is directly suitable for expression in any host.

The nucleic acid molecule is then preferably placed in operable linkage with suitable control sequences, as described above, to form an expression unit containing the protein open reading frame. The expression unit is used to transform a suitable host and the transformed host is cultured under conditions that allow the production of the recombinant protein. Optionally the recombinant protein is isolated from the medium or from the cells; recovery and purification of the protein may not be necessary in some instances where some impurities may be tolerated.

Each of the foregoing steps can be done in a variety of ways. For example, the desired coding sequences may be obtained from genomic fragments and used directly in appropriate hosts. The construction of expression vectors that are operable in a variety of hosts is accomplished using appropriate replicons and control sequences, as set forth above. The control sequences, expression vectors, and transformation methods are dependent on the type of host cell used to express the gene and were discussed in detail earlier. Suitable restriction sites can, if not normally available, be added to the ends of the coding sequence so as to provide an excisable gene to insert into these vectors. A skilled artisan can readily adapt any host/expression system known in the art for use with the nucleic acid molecules of the invention to produce recombinant protein.

G. Methods to Identify Binding Partners

Another embodiment of the present invention provides methods for use in isolating and identifying binding partners of proteins of the invention. In detail, a protein of the invention is mixed with a potential binding partner or an extract or fraction of a cell under conditions that allow the association of potential binding partners with the protein of the invention. After mixing, peptides, polypeptides, proteins or other molecules that have become associated with a protein of the invention are separated from the mixture. The binding partner that bound to the protein of the invention can then be removed and further analyzed. To identify and isolate a binding partner, the entire protein, for instance a ICACC protein of SEQ ID No.2, SEQ ID No. 4 or SEQ ID NO: 6 can be used. Alternatively, a fragment of the protein or a

membrane fragment containing the protein may be used.

As used herein, a cellular extract refers to a preparation or fraction which is made from a lysed or disrupted cell. The preferred source of cellular extracts are cells derived from human tissues or cells.

5 A variety of methods can be used to obtain an extract of a cell. Cells can be disrupted using either physical or chemical disruption methods. Examples of physical disruption methods include, but are not limited to, sonication and mechanical shearing. Examples of chemical lysis methods include, but are not limited to, detergent lysis and enzyme lysis. A skilled artisan can readily adapt methods for preparing cellular extracts in order to obtain
10 extracts for use in the present methods.

Once an extract of a cell is prepared, the extract is mixed with the protein of the invention under conditions in which association of the protein with the binding partner can occur. A variety of conditions can be used, the most preferred being conditions that closely resemble conditions found in the cytoplasm of a human cell. Features such as osmolarity, pH,
15 temperature, and the concentration of cellular extract used, can be varied to optimize the association of the protein with the binding partner.

After mixing under appropriate conditions, the bound complex is separated from the mixture. A variety of techniques can be utilized to separate the mixture. For example, antibodies specific to a protein of the invention can be used to immunoprecipitate the binding
20 partner complex. Alternatively, standard chemical separation techniques such as chromatography and density/sediment centrifugation can be used.

After removal of non-associated cellular constituents found in the extract, the binding partner can be dissociated from the complex using conventional methods. For example, dissociation can be accomplished by altering the salt concentration or pH of the mixture.
25 To aid in separating associated binding partner pairs from the mixed extract, the protein of the invention can be immobilized on a solid support. For example, the protein can be attached to a nitrocellulose matrix or acrylic beads. Attachment of the protein to a solid support aids in separating peptide/binding partner pairs from other constituents found in the extract. The identified binding partners can be either a single protein or a complex made up
30 of two or more proteins. Alternatively, binding partners may be identified using a Far-Western assay according to the procedures of Takayama *et al.* (1997) *Methods Mol. Biol.*

69:171-84 or Sauder *et al.* J Gen. Virol. 77(5):991-6 or identified through the use of epitope tagged proteins or GST fusion proteins.

Alternatively, the nucleic acid molecules of the invention can be used in a yeast two-hybrid system, preferably systems for screening binding partners of membrane proteins. The yeast two-hybrid system has been used to identify other protein partner pairs and can readily be adapted to employ the nucleic acid molecules herein described.

H. Methods to Identify Agents that Modulate the Expression a Nucleic Acid Encoding and ICACC Protein.

Another embodiment of the present invention provides methods for identifying agents that modulate the expression of a nucleic acid encoding a protein of the invention such as a protein having the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:6. Such assays may utilize any available means of monitoring for changes in the expression level of the nucleic acids of the invention. As used herein, an agent is said to modulate the expression of a nucleic acid of the invention, for instance a nucleic acid encoding the protein having the sequence of SEQ ID NO:2 or SEQ ID NO:6 if it is capable of up- or down-regulating expression of the nucleic acid in a cell.

Agents of the invention may relate to antisense or gene therapy. It is now known in the art that altered DNA molecules can be tailored to provide a selected effect, when provided as antisense or gene therapy. The native DNA segment coding for ICACC-1 has two strands; a sense strand and an antisense strand held together by hydrogen bonds. The mRNA coding for the receptor has a nucleotide sequence identical to the sense strand, with the expected substitution of thymidine by uridine. Thus, based upon the knowledge of the receptor sequence, synthetic oligonucleotides can be synthesized. These oligonucleotides can bind to the DNA and RNA coding for ICACC-1. The active fragments of the invention, which are complementary to mRNA and the coding strand of DNA, are usually at least about 15 nucleotides, more usually at least 20 nucleotides, preferably 30 nucleotides and more preferably may be 50 nucleotides or more. The binding strength between the sense and antisense strands is dependent upon the total hydrogen bonds. Therefore, based upon the total number of bases in the mRNA, the optimal length of the oligonucleotide sequence may be easily calculated by the skilled artisan.

The sequence may be complementary to any portion of the sequence of the mRNA. For example, it may be proximal to the 5'-terminus or capping site or downstream from the capping site, between the capping site and the initiation codon and may cover all or only a portion of the non-coding region or the coding region. The particular site(s) to which the antisense sequence binds will vary depending upon the degree of inhibition desired, the uniqueness of the sequence, the stability of the antisense sequence, etc.

In the practice of the invention, expression of ICACC-1 is down-regulated by administering an effective amount of antisense oligonucleotide sequences described above. The oligonucleotide compounds of the invention bind to the mRNA coding for human ICACC-1 thereby inhibiting expression (translation) of these proteins. The isolated DNA sequences, containing various mutations such as point mutations, insertions, deletions or spliced mutations of ICACC-1 are useful in gene therapy as well.

In one assay format for agents, cell lines that contain reporter gene fusions between the open reading frame and any assayable fusion partner may be prepared. Numerous assayable fusion partners are known and readily available including the firefly luciferase gene and the gene encoding chloramphenicol acetyltransferase (Alam *et al.* (1990) *Anal. Biochem.* 188:245-254). Cell lines containing the reporter gene fusions are then exposed to the agent to be tested under appropriate conditions and time. Differential expression of the reporter gene between samples exposed to the agent and control samples identifies agents which modulate the expression of a nucleic acid encoding an ICACC-1 protein.

Additional assay formats may be used to monitor the ability of the agent to modulate the expression of a nucleic acid encoding a protein of the invention, such as the protein having SEQ ID NO:2 or SEQ ID NO:6. For instance, mRNA expression may be monitored directly by hybridization to the nucleic acids of the invention. Cell lines are exposed to the agent to be tested under appropriate conditions and time and total RNA or mRNA is isolated by standard procedures such those disclosed in Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 2nd Ed. Clod Spring Harbor Laboratory Press, 1989).

Probes to detect differences in RNA expression levels between cells exposed to the agent and control cells may be prepared from the nucleic acids of the invention. It is preferable, but not necessary, to design probes which hybridize only with target nucleic acids under conditions of high stringency. Only highly complementary nucleic acid hybrids form under conditions

of high stringency. Accordingly, the stringency of the assay conditions determines the amount of complementarity which should exist between two nucleic acid strands in order to form a hybrid. Stringency should be chosen to maximize the difference in stability between the probe:target hybrid and potential probe:non-target hybrids.

5 Probes may be designed from the nucleic acids of the invention through methods known in the art. For instance, the G+C content of the probe and the probe length can affect probe binding to its target sequence. Methods to optimize probe specificity are commonly available in Sambrook *et al.* (*Molecular Cloning: A Laboratory Approach*, Cold Spring Harbor Press, NY, 1989) or Ausubel *et al.* (*Current Protocols in Molecular Biology*, Greene Publishing
10 Co., NY, 1995).

Hybridization conditions are modified using known methods, such as those described by Sambrook *et al.* and Ausubel *et al.* as required for each probe. Hybridization of total cellular RNA or RNA enriched for polyA RNA can be accomplished in any available format. For instance, total cellular RNA or RNA enriched for polyA RNA can be affixed to a solid
15 support and the solid support exposed to at least one probe comprising at least one, or part of one of the sequences of the invention under conditions in which the probe will specifically hybridize. Alternatively, nucleic acid fragments comprising at least one, or part of one of the sequences of the invention can be affixed to a solid support, such as a porous glass wafer. The glass wafer can then be exposed to total cellular RNA or polyA RNA from a sample
20 under conditions in which the affixed sequences will specifically hybridize. Such glass wafers and hybridization methods are widely available, for example, those disclosed by Beattie (WO 95/11755). By examining for the ability of a given probe to specifically hybridize to an RNA sample from an untreated cell population and from a cell population exposed to the agent, agents which up or down regulate the expression of a nucleic acid
25 encoding an ICACC protein, preferably an ICACC-1 protein, are identified.

Hybridization for qualitative and quantitative analysis of mRNAs may also be carried out by using a RNase Protection Assay (i.e., RPA, see Ma *et al.* (1996) *Methods* 10: 273-238). Briefly, an expression vehicle comprising cDNA encoding the gene product and a phage specific DNA dependent RNA polymerase promoter (e.g., T7, T3 or SP6 RNA polymerase)
30 is linearized at the 3' end of the cDNA molecule, downstream from the phage promoter, wherein such a linearized molecule is subsequently used as a template for synthesis of a

labeled antisense transcript of the cDNA by *in vitro* transcription. The labeled transcript is then hybridized to a mixture of isolated RNA (i.e., total or fractionated mRNA) by incubation at 45°C overnight in a buffer comprising 80% formamide, 40 mM Pipes, pH 6.4, 0.4 M NaCl and 1 mM EDTA. The resulting hybrids are then digested in a buffer comprising 40 µg/ml
5 ribonuclease A and 2 µg/ml ribonuclease. After deactivation and extraction of extraneous proteins, the samples are loaded onto urea/polyacrylamide gels for analysis.

In another assay format for agents which effect the expression of the instant gene products, cells or cell lines would first be identified which express said gene products physiologically (e.g., see the Figures for tissue distribution). Cell and/or cell lines so
10 identified would be expected to comprise the necessary cellular machinery such that the fidelity of modulation of the transcriptional apparatus is maintained with regard to exogenous contact of agent with appropriate surface transduction mechanisms and/or the cytosolic cascades. Further, such cells or cell lines would be transduced or transfected with an expression vehicle (e.g., a plasmid or viral vector) construct comprising an operable non-
15 translated 5'-promoter containing end of the structural gene encoding the instant gene products fused to one or more antigenic fragments, which are peculiar to the instant gene products, wherein said fragments are under the transcriptional control of said promoter and are expressed as polypeptides whose molecular weight can be distinguished from the naturally occurring polypeptides or may further comprise an immunologically distinct tag.
20 Such a process is well known in the art (see Maniatis). Cells may be exposed to IL-9.

Cells or cell lines transduced or transfected as outlined above would then be contacted with agents under appropriate conditions; for example, the agent comprises a pharmaceutically acceptable excipient and is contacted with cells comprised in an aqueous physiological buffer such as phosphate buffered saline (PBS) at physiological pH, Eagles
25 balanced salt solution (BSS) at physiological pH, PBS or BSS comprising serum or conditioned media comprising PBS or BSS and/or serum incubated at 37° C. Said conditions may be modulated as deemed necessary by one of skill in the art. Subsequent to contacting the cells with the agent, said cells will be disrupted and the polypeptides of the disruptate are fractionated such that a polypeptide fraction is pooled and contacted with an
30 antibody to be further processed by immunological assay (e.g., ELISA, immunoprecipitation or Western blot). The pool of proteins isolated from the "agent contacted" sample will be

compared with a control sample where only the excipient is contacted with the cells and an increase or decrease in the immunologically generated signal from the "agent contacted" sample compared to the control will be used to distinguish the effectiveness of the agent.

I. Methods to Identify Agents that Modulate at Least One Activity of an ICACC

5 Protein.

Another embodiment of the present invention provides methods for identifying agents that modulate at least one activity of a protein of the invention, such as a protein having the amino acid sequence of SEQ ID No.2, SEQ ID NO:4 or SEQ ID No.6 and preferably, an ICACC-1 protein. Such methods or assays may utilize any means of monitoring or detecting
10 the desired activity.

Specific assays may be based on monitoring the cellular functions of ICACC-1. Antagonists of the invention include those molecules that interact or bind to ICACC-1 and inactivate this receptor. To identify other allosteric, inverse or weak antagonists of the invention, one may test for binding to ICACC-1. The present invention includes antagonists
15 of ICACC-1 that block activation of this receptor. Antagonists are compounds that are themselves devoid of pharmacological activity but cause effects by preventing the action of an agonist. To identify an antagonist of the invention, one may test for competitive binding with natural ligands of ICACC-1. Assays of antagonistic binding and activity can be derived from monitoring ICACC-1 functions for down-regulation as described herein and in the cited
20 literature. The binding of the antagonist may involve all known types of interactions including ionic forces, hydrogen bonding, hydrophobic interactions, van der Waals forces and covalent bonds. In many cases, bonds of multiple types are important in the interaction of an agonist or antagonist with a molecule like ICACC-1.

In a further embodiment, these compounds may be analogues of ICACC-1 or its ligands.
25 ICACC-1 analogues may be produced by point mutations in the isolated DNA sequence for the gene, nucleotide substitutions and/or deletions which can be created by methods that are all well described in the art (Simoncsits *et al.*, 1994). This invention also includes spliced variants of ICACC-1 and discloses isolated nucleic acid sequences of ICACC-1, which contain deletions of one or more of its exons. The term "spliced variants" as used herein
30 denotes a purified and isolated DNA molecule encoding human ICACC-1 comprising at least

one exon. There is no evidence of naturally expressed spliced mutants in the art. It must be understood that these exons may contain various point mutations.

Structure-activity relationships may be used to modify the antagonists of the invention. For example, the techniques of X-ray crystallography and NMR may be used to make
5 modifications of the invention. For example, one can create a three-dimensional structure of human ICACC-1 that can be used as a template for building structural models of deletion mutants using molecular graphics. These models can then be used to identify and construct a ligand for ICACC-1 which alters normal chloride channel function. In still another embodiment, these compounds may also be used as dynamic probes for ICACC-1 structure
10 and to develop ICACC-1 antagonists using cell lines or other suitable means of assaying ICACC-1 activity.

In addition, this invention also provides compounds that prevent the synthesis or reduce the biologic stability of ICACC-1. Biologic stability is a measure of the time between the synthesis of the molecule and its degradation. For example, the stability of a protein, peptide
15 or peptide mimetic (Kauvar, 1996) therapeutic may be prolonged by using D-amino acids or shortened by altering its sequence to make it more susceptible to enzymatic degradation.

In another embodiment, antagonists of the invention are antibodies to ICACC-1. The antibodies to ICACC-1 may be either monoclonal or polyclonal, made using standard techniques well known in the art (See Harlow & Lane's Antibodies: A Laboratory Manual,
20 Cold Spring Harbor Laboratory Press, 1988). They can be used to block ICACC-1 activation by binding to extracellular regions of the protein required for ligand binding or activation. In one embodiment, the antibodies interact with ICACC-1, in another they interact with the ligands for ICACC-1. The ICACC-1 used to elicit these antibodies can be the ICACC-1 protein or any of the ICACC-1 variants or fragments discussed above. Antibodies are also
25 produced from peptide sequences of ICACC-1 using standard techniques in the art (see Protocols in Immunology, John Wiley & Sons, 1994).

In one assay format, the relative amounts of ICACC-1 protein of the invention between a cell population that has been exposed to the agent to be tested compared to an un-exposed control cell population may be assayed. In this format, probes such as specific antibodies are
30 used to monitor the differential expression of the protein in the different cell populations. Cell lines or populations are exposed to the agent to be tested under appropriate conditions

and time. Cellular lysates may be prepared from the exposed cell line or population and a control, unexposed cell line or population. The cellular lysates are then analyzed with the probe.

Antibody probes are prepared by immunizing suitable mammalian hosts in appropriate immunization protocols using the peptides, polypeptides or proteins of the invention if they are of sufficient length, or, if desired, or if required to enhance immunogenicity, conjugated to suitable carriers. Methods for preparing immunogenic conjugates with carriers such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be effective; in other instances linking reagents such as those supplied by Pierce Chemical Co., Rockford, IL, may be desirable to provide accessibility to the hapten. The hapten peptides can be extended at either the amino or carboxy terminus with a Cys residue or interspersed with cysteine residues, for example, to facilitate linking to a carrier. Administration of the immunogens is conducted generally by injection over a suitable time period and with use of suitable adjuvants, as is generally understood in the art. During the immunization schedule, titers of antibodies are taken to determine adequacy of antibody formation.

While the polyclonal antisera produced in this way may be satisfactory for some applications, for pharmaceutical compositions, use of monoclonal preparations is preferred. Immortalized cell lines which secrete the desired monoclonal antibodies may be prepared using the standard method of Kohler and Milstein or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the peptide hapten, polypeptide or protein. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either *in vitro* or by production in ascites fluid.

The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can be used as antagonists, as well as the intact antibodies. Use of immunologically reactive fragments, such as the Fab, Fab', of F(ab')₂ fragments is often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Antibody regions that bind specifically to the desired regions of the protein can also be produced in the context of chimeras with multiple species origin.

Agents that are assayed in the above method can be randomly selected or rationally selected
5 or designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences involved in the association of the a protein of the invention alone or with its associated substrates, binding partners, etc. An example of randomly selected agents is the use a chemical library or a peptide combinatorial library, or a growth broth of an organism.

10 As used herein, an agent is said to be rationally selected or designed when the agent is chosen on a nonrandom basis which takes into account the sequence of the target site and/or its conformation in connection with the agent's action.

The agents of the present invention can be, as examples, peptides, small molecules, vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that
15 there is no limit as to the structural nature of the agents of the present invention.

The peptide agents of the invention can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems.
20 The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

Another class of agents of the present invention are antibodies immunoreactive with critical positions of proteins of the invention. Antibody agents are obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions
25 of the protein intended to be targeted by the antibodies.

J. Uses for Agents that Modulate at Least One Activity of an ICACC Protein.

Further evidence defining the role of ICACC-1 in the pathogenesis of atopic allergy, bronchial hyperresponsiveness, asthma and related disorders is derived directly from the Applicants observation that IL-9 selectively induces ICACC-1. Thus, the pleiotropic role for
30 IL-9, which is important to a number of antigen induced responses is dependent in part, on

the up-regulation of ICACC-1 in cells critical to atopic allergy. When the functions of IL-9 are down-regulated by antibody pretreatment prior to aerosol challenge with antigen, animals can be completely protected from the antigen induced responses. These responses include: bronchial hyperresponsiveness, eosinophilia and elevated cell counts in bronchial lavage, histologic changes in lung associated with inflammation and elevated serum IgE. The suppression of IL-9 and asthmatic-like responses is associated with down regulated expression of ICACC-1 (Figure 13). Thus, treatment of such responses, which underlie the pathogenesis of atopic allergy and characterize allergic inflammation associated with this disorder, by down-regulating ICACC-1, is within the scope of this invention.

10 The involvement of chloride channels in IL-9 biologic responses is addressed by in vitro primary lung cultures that produce secreted eotaxin protein upon IL-9 stimulation (Figure 17). The treatment of these cultures with known chloride channel inhibitors results in suppression of the IL-9 induced eotaxin response (Figure 18) and thus provides an assay for screening for ICACC-1 inhibitors. In another embodiment cell lines in which ICACC-1 expression vectors are introduced can be used to screen for specific chloride channel inhibitors.

Applicants also teach the down-regulation of ICACC-1 by administering antagonists of ICACC-1. The skilled artisan will recognize that all molecules containing the requisite three-dimensional structural conformation critical for activation of, or ligand binding to ICACC-1 are within the scope of this invention.

The demonstration of an IL-9 sequence associated with an asthma-like phenotype and one associated with the absence of an asthma-like phenotype, indicates that the inflammatory response to antigen in the lung is IL-9 dependent. Down-regulating ICACC-1, which is selectively induced downstream in the IL-9 pathway, will therefore protect against this antigen induced response.

In addition to the direct regulation of the ICACC-1 gene, this invention also encompasses methods of inhibiting the intracellular signaling by ICACC-1. It is known in the art that highly exergonic phosphoryl-transfer reactions are catalyzed by various enzymes known as kinases. In other words, a kinase transfers phosphoryl groups between ATP and a metabolite. Included within the scope of this invention are specific inhibitors of protein kinases. Thus, inhibitors of these kinases are useful in the down-regulation of ICACC-1 and

are therefore useful in the treatment of atopic allergies and asthma.

In still another aspect of the invention, surprisingly, aminosterol compounds were found to be useful in the inhibition of ICACC-1 induction by IL-9. Aminosterol compounds which are useful in this invention are described in U.S. Patent Application, Serial No. 08/290,826
5 and its related applications 08/416,883 and 08/478,763 as well as in 08/483,059 and its related applications 08/483,057, 08/479,455, 08/479,457, 08/475,572, 08/476,855, 08/474,799 and 08/487,443, which are specifically incorporated herein by reference in their entirety.

While a therapeutic potential for ICACC-1 down-regulation has been identified,
10 Applicants have also recognized a therapeutic potential for up-regulation of ICACC-1 as well. Patients with cystic fibrosis are hampered by lung disease characterized by thick secretions, which cause airway obstruction and subsequent colonization and infection by inhaled pathogenic microorganisms (Eng *et al.*, 1996). Airway epithelia from cystic fibrosis patients exhibit a broad spectrum of ion transport properties that differ from normal,
15 including not only defective cAMP-mediated chloride secretion, but also increased sodium absorption and increased calcium-mediated chloride secretion (Johnson *et al.*, 1995). Restoration of overall chloride secretion in primary cystic fibrosis airway epithelial cells leads to correction of sodium hyperabsorption and normal airway epithelial cell function (Johnson *et al.*, 1995). Applicants therefore provide a method for treating cystic fibrosis by
20 further increasing calcium-dependent chloride secretion in these cells through up-regulation of ICACC-1 activity in airway epithelia. In this manner, the decrease in chloride secretion due to the defect in cAMP-mediated chloride secretion is compensated for through up-regulation of ICACC-1. The result being a restoration of the cellular chloride gradient and normal airway epithelial cell function. In another indication, up regulation of ICACC-1 will
25 be useful for treating autoimmune associated diseases such as IBD.

As provided in the Examples, the proteins and nucleic acids of the invention, such as the ICACC-1 proteins having the amino acid sequence of SEQ ID NOS: 2 or 6, are induced by IL-9. Agents that modulate or down-regulate the expression of the protein or agents such as agonists or antagonists of at least one activity of the protein may be used to modulate
30 biological and pathologic processes associated with the protein's function and activity. As used herein, a subject can be any mammal, so long as the mammal is in need of

modulation of a pathological or biological process mediated by a protein of the invention.

The term "mammal" is meant an individual belonging to the class Mammalia. The invention is particularly useful in the treatment of human subjects.

Pathological processes refer to a category of biological processes which produce a deleterious effect. For example, expression of a protein of the invention may be associated with atopic allergy, asthma and/or cystic fibrosis. As used herein, an agent is said to modulate a pathological process when the agent reduces the degree or severity of the process. For instance, atopic allergy, asthma and/or cystic fibrosis may be prevented or disease progression modulated by the administration of agents which reduce or modulate in some way the expression or at least one activity of a protein of the invention.

The agents of the present invention can be provided alone, or in combination with other agents that modulate a particular pathological process. For example, an agent of the present invention can be administered in combination with anti-asthma agents. As used herein, two agents are said to be administered in combination when the two agents are administered simultaneously or are administered independently in a fashion such that the agents will act at the same time.

The agents of the present invention can be administered via parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route or directly to the lungs. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

The compounds used in the method of treatment of this invention may be administered systemically or topically, depending on such considerations as the condition to be treated, need for site-specific treatment, quantity of drug to be administered and similar considerations.

Topical administration may be used. Any common topical formation such as a solution, suspension, gel, ointment or salve and the like may be employed. Preparation of such topical formulations are well described in the art of pharmaceutical formulations as exemplified, for example, by Remington's Pharmaceutical Sciences. For topical application, these compounds could also be administered as a powder or spray, particularly in aerosol form. The active ingredient may be administered in pharmaceutical compositions adapted for systemic

administration. As is known, if a drug is to be administered systemically, it may be confectioned as a powder, pill, tablet or the like or as a syrup or elixir for oral administration. For intravenous, intraperitoneal or intra-lesional administration, the compound will be prepared as a solution or suspension capable of being administered by injection. In certain cases, it may be useful to formulate these compounds in suppository form or as an extended release formulation for deposit under the skin or intramuscular injection. In a preferred embodiment, the compounds of this invention may be administered by inhalation. For inhalation therapy the compound may be in a solution useful for administration by metered dose inhalers or in a form suitable for a dry powder inhaler.

10 An effective amount is that amount which will down-regulate ICACC-1. A given effective amount will vary from condition to condition and in certain instances may vary with the severity of the condition being treated and the patient's susceptibility to treatment. Accordingly, a given effective amount will be best determined at the time and place through routine experimentation. However, it is anticipated that in the treatment of atopic allergy and asthma-related disorders in accordance with the present invention, a formulation containing 15 between 0.001 and 5 percent by weight, preferably about 0.01 to 1%, will usually constitute a therapeutically effective amount. When administered systemically, an amount between 0.01 and 100 mg per kg body weight per day, but preferably about 0.1 to 10 mg/kg, will effect a therapeutic result in most instances.

20 The invention also includes pharmaceutical compositions comprising the compounds of the invention together with a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can 25 also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, Mack Publishing Company, 1995. In addition to the pharmacologically active agent, the compositions of the present invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active 30 compounds into preparations which can be used pharmaceutically for delivery to the site of

action. Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection
5 suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for delivery into the cell.

10 The pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulations may be used simultaneously to achieve systemic administration of the active ingredient.

Suitable formulations for oral administration include hard or soft gelatin capsules, pills,
15 tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

In practicing the methods of this invention, the compounds of this invention may be used alone or in combination, or in combination with other therapeutic or diagnostic agents. In certain preferred embodiments, the compounds of this invention may be coadministered
20 along with other compounds typically prescribed for these conditions according to generally accepted medical practice. The compounds of this invention can be utilized *in vivo*, ordinarily in mammals, preferably in humans.

In still another embodiment, the compounds of the invention may be coupled to chemical moieties, including proteins that alter the functions or regulation of ICACC-1 for therapeutic
25 benefit in atopic allergy and asthma (Kreitman *et al.*, 1994). These proteins may include in combination other inhibitors of cytokines and growth factors including anti-IL-4, anti-IL-5, anti-IL-3, anti-IL-2, anti-IL-13, anti-IL-11 and anti-IL-10 that may offer additional therapeutic benefit in atopic allergy and asthma. In addition, the molecules of the invention may also be conjugated through phosphorylation to biotinylate, thioate, acetylate, iodinate
30 using any of the cross-linking reagents well known in the art.

K. Diagn stics

Also included in the invention are methods of diagnosing susceptibility to atopic allergy and related disorders and for treating these disorders based on the relationship between IL-9, its receptor and ICACC-1.

- 5 These disorders also include the monitoring of ICACC-1 gene expression for the diagnosis of autoimmune disease of the bowel such as inflammatory bowel disease (IBD). In the case of IBD the lack or suppression of ICACC-1 gene expression would be a diagnostic marker for the disease and the ability to follow ICACC-1 levels would aid in monitoring treatment.
- 10 One diagnostic embodiment involves the recognition of variations in the DNA sequence of ICACC-1. One method involves the introduction of a nucleic acid molecule (also known as a probe) having a sequence complementary to ICACC-1 of the invention under sufficient hybridizing conditions, as would be understood by those in the art. In one embodiment, the sequence will bind specifically to one allele of ICACC-1 or a fragment thereof and in another
- 15 embodiment will bind to both alleles. Another method of recognizing DNA sequence variation associated with these disorders is direct DNA sequence analysis by multiple methods well known in the art (Ott, 1991). Another embodiment involves the detection of DNA sequence variation in the ICACC-1 gene associated with these disorders (Schwengel *et al.*, 1993; Sheffield *et al.*, 1993; Orita *et al.*, 1989; Sarkar *et al.*, 1992; Cotton, 1989). These
- 20 include the polymerase chain reaction, restriction fragment length polymorphism analysis and single stranded conformational analysis.

- The practice of the present invention will employ the conventional terms and techniques of molecular biology, pharmacology, immunology and biochemistry that are within the ordinary skill of those in the art. For example, see Sambrook *et al.*, Molecular Cloning: A
- 25 Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, 1985.

- Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out preferred embodiments of the present
- 30 invention, and are not to be construed as limiting in any way the remainder of the disclosure.

Examples

Example 1: *cDNA Difference Analysis of IL-9 expressed genes.*

Lungs extracted from transgenic IL-9 mice (Tg5) were used to isolate IL-9 induced genes. Tg5 is a FVB mouse overexpressing the IL-9 gene as previously described (Renauld
5 *et al.*, 1994). This strain has been shown to overexpress IL-9 in most tissues of the mouse. In order to identify specific IL-9 induced genes, suppressive PCR cDNA difference analysis was performed on mRNA from lungs of Tg5 mice and parental FVB mice using a commercially available PCR-select cDNA subtraction kit (Clontech).

cDNA synthesis. Total RNA was prepared from lungs of FVB and Tg5 mice using Trizol
10 as described by the manufacturer (Gibco/BRL). Lungs were removed from euthanized mice and frozen in liquid nitrogen. Frozen lungs were then placed in Trizol and pulverized using a tissue grinder. Polyadenylated RNA was purified from total RNA with oligo(dT) cellulose columns (Pharmacia). Double stranded cDNA was prepared using Superscript II reverse transcriptase and an oligo(dT) primer as suggested by the manufacturer (Clontech). cDNA
15 was then prepared by phenol-chloroform extraction and ethanol precipitation. Products were resuspended in nuclease-free water and analyzed on agarose gels to determine quality of products as described below.

cDNA difference analysis protocol. Differential cDNA analysis of Tg5 and FVB lungs was carried out following the manufacturers protocol (Clontech) as depicted in Figure 1.
20 The results of the subtraction between the cDNA of these lungs resulted in the generation of 1200 recombinant clones. Analysis of these clones revealed multiples of several species, each accounting for 2-5% of the library. The most prominent transcript in the library was the IL-9 cDNA which served as a control for the efficiency of subtraction since it was a subtraction between an IL-9 constitutively expressing mouse (Tg5) and its parental control.
25 Another cDNA which was found in multiple copies (represented 3% of library) was a novel calcium activated chloride channel which is described below.

Example 2: *Identification of the murine ICACC-1 cDNA in the lung of IL-9 transgenic mice.*

ICACC-1 probes as described in Example 1 were used to probe a murine lung cDNA library (Clontech) according to the manufacturers recommendation. One million

recombinant clones were screened and several overlapping phage were identified. Subsequent screens enabled identification and isolation of a single plaque containing a phagemid which was then transformed into a double-stranded plasmid by phage rescue according to the manufacturers protocol. Recombinant clones were prepared and sequenced
5 using primers directed to the plasmid vector as well as internal sequences identified from the partially subtracted probe. Clones were then aligned and contiged to generate the full-length sequence.

The 2931 bp cDNA isolated contained an open reading frame encoding a protein of 925 amino acids. Figure 2 shows the nucleotide and amino acid sequence of the murine ICACC-
10 1 cDNA. A nucleotide BLAST (Altschul *et al.*, 1990) database search of GenBank with the full length cDNA revealed that it was similar to the bovine chloride channel protein. Figure 3 shows an alignment to the bovine calcium activated chloride channel cDNA. Motif analysis of the encoded polypeptide demonstrated several features such as multiple transmembrane regions and glycosylation sites. The primary sequence of murine ICACC-1 was used to
15 perform an EST database search and several undescribed human ESTs were found to be homologous to small portions of the novel cDNA. Figure 4A and 4B show the sequences of the human ICACC-1 and ICACC-2 genes. Both full length human ICACC sequences were obtained by screening a human cDNA library.

Example 3: ICACC-1 is induced in vivo by IL-9 in murine cells.

20 To confirm that ICACC-1 is induced by IL-9 in the lung, RNA from the lungs of Tg5 and FVB mice were isolated as described in Example 1. cDNA was generated using random hexamers (Pharmacia) and Superscript II (Gibco/BRL) as suggested by the manufacturer. Message was analyzed by PCR as described (Nicolaidis *et al.*, 1995) and via Northern blot. Primers used to generate murine ICACC-1 message were; sense 5'-
25 CCAGATCCACACCAAAACGAGAAG-3' (SEQ ID NO:7) (nucleotides 689-712) and antisense 5'-CACTGTCAAAGGTCACCATCCCGA-3' (SEQ ID NO:8) (nucleotides 1041-1064) which produce a gene product of 376 bp. DHFR was assayed as an internal control to measure for cDNA integrity using primers previously described (Nicolaidis *et al.*, 1991). Amplification conditions used were 95°C for 30 seconds, 58°C for 1.5 minutes and 72°C for
30 1.5 minutes for 35 cycles. For Northern blot analysis, total RNA derived from Tg5 or FVB

lungs was electrophoresed on 1.5% formaldehyde gels, transferred to nylon membranes and probed with a DNA fragment comprising the murine ICACC-1 cDNA.

The results of the expression studies demonstrated that ICACC-1 is specifically expressed in the lung of the IL-9 transgenic mouse but not in the parental strain (Figure 6). This data
5 demonstrated a direct effect of IL-9 on ICACC-1 expression in the lung, where IL-9 responsive cells contained within the lung express ICACC-1.

Example 4: ICACC-1 expression can be induced in the murine lung by IL-9.

ICACC-1 gene expression was assessed in vivo using the C57B6 mouse (bronchial hyporesponsive) which does not express detectable levels of IL-9 and the DBA mouse
10 (bronchial hyperresponsive) which expresses robust levels of IL-9 (Nicolaidis *et al.*, 1997). RT-PCR and Northern blot analysis of ICACC-1 from these lungs demonstrated that ICACC-1 was expressed in the lung of mice which naturally express high levels of IL-9 (DBA) but not in those with low levels of IL-9 (C57B6) (Figure 7).

To confirm that the expression of IL-9 was critically related to the expression of ICACC-
15 1 and to control for genetic background specifically, recombinant murine IL-9 was introduced into the lung of murine strain C57B6. Recombinant IL-9 was instilled into the trachea of anesthetized mice by addition of 50 µl of a 0.1 mg/ml IL-9 solution or vehicle alone (0.1% bovine serum albumin) daily for ten days. After ten days, the mice were euthanized and lungs extracted for either RNA expression analysis using Trizol as described
20 by the manufacturer (Gibco/BRL) or Western blot analysis to determine levels of IL-9 instilled. The Western blot analysis for IL-9 demonstrated that direct addition of IL-9 to the lung resulted in an increase of overall amount of IL-9 in the lung while none was observed in the mouse instilled with vehicle alone.

Expression of ICACC-1 RNA was measured as described in Example 3. RT-PCR
25 analysis for ICACC-1 RNA expression indicated that expression increased when recombinant IL-9 was administered to the lungs of the C57B6 mice, while no expression was observed in the lungs of mice treated with vehicle only (Figure 8). This data demonstrates a direct role of IL-9 on inducing ICACC-1 expression in the lung.

Example 5: *Tissue distribution of ICACC-1 in mice.*

To address the possibility that ICACC-1 expression occurs only in the presence of IL-9 expression, various organs were extracted from Tg5 mice and analyzed for RNA expression via Northern blot. BALBc mice were used as a control because they express low levels of IL-9 in the lung when compared to Tg5 mice. Tissue blots derived from BALBc murine organs were commercially obtained (Clontech) while tissue blots for Tg5 murine organs were prepared by extracting organs followed by freezing in liquid nitrogen. Total RNA was extracted from each of these organs using Trizol as described by the manufacturer (Gibco/BRL). RNA was gel electrophoresed and analyzed as described in Example 4.

10 Lanes were standardized by probing with β -actin as an internal control.

Tissue blots were probed using a DNA fragment comprising the ICACC-1 cDNA. As shown in Figure 9 (bottom), no signal was observed in any of the tissues present on blots from normal mice. Analysis of ICACC-1 expression in Tg5 organs revealed expression in the lung, lymph node, colon, spleen, stomach, ovary and uterus (Figure 9, top). This data demonstrated that ICACC-1 is expressed in several tissues in mice overexpressing IL-9 but not in those with low IL-9 levels. This data suggests that ICACC-1 may play a role in the physiology of these organs in response to IL-9.

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Example 5A: *Induction of ICACC-1 in the lung by exposure to antigen.*

Antigen sensitization and phenotyping of animals was carried out as previously described (McLane, MP, *et al.* Am. J. Respir. Cell Mol. Biol. 19:713-720, 1998). Briefly, Balb/C mice were intranasally exposed to *Aspergillus fumigatus* for 3-4 weeks. One day after the final exposure, antigen exposed mice and naïve controls were phenotyped for bronchial hyperresponsiveness (BHR) and cellularity in the airway. After phenotyping, organs were removed and total RNA was prepared as described in Example 5 and ICACC-1 expression was assessed in naïve and antigen treated tissues. As shown in Figure 10, antigen exposed Balb/C mice had a significant increase in BHR (Figure 10A) and inflammatory cell influx (the majority being eosinophils) as compared to controls (Figure 10B). These features are very similar to clinical human asthma, and reinforce the notion that this is a relevant model to study molecular mechanisms and pharmaceutical target discovery for the development of asthma drugs. ICACC-1 gene expression was tightly associated with the asthmatic-like lung

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where robust expression was found in the antigen treated lung (bottom panel, Figure 11), while no expression was found in the naïve "normal" lung (top panel, Figure 11). These data suggest that: 1) ICACC-1 is a potential therapeutic target for the treatment of asthma, and 2) inhibiting the expression or function of ICACC-1 will result in no toxic effects to the lung.

Example 5B: *Inhibition of antigen induced induction of ICACC-1 in the lung with anti-IL-9*

IL-9 is a major mediator of the asthmatic response in man and mouse models of asthma (Nicolaidis, *et al.* Proc. Natl. Acad. Sci. 94:13175-13180, 1997; McLane, MP, *et al.* Am. J. Respir. Cell Mol. Biol. 19:713-720, 1998; Temann *et al.*, J. Exp. Med. 188:1307-1320, 1998; Levitt and Nicolaidis, Emerg. Thera. Targets 3:1-11, 1999). The use of IL-9 blocking antibodies in antigen exposed mice suppresses the asthmatic-like phenotype (bronchial hyperresponsiveness and influx of inflammatory cells such as eosinophils). (B6D2)F1 mice were exposed to *Aspergillus fumigatus* antigen as described in Example 5A on day 0, 7, 14, 21, and 22. A subset of mice were also treated with 200 µg of anti-mIL9 (Pharmingen hamster antimouse IL-9) intra nasally on day 0, 7, 14, and 21; or an isotype control Ig; or saline alone. All mice and naïve controls were phenotyped for BHR and BAL analysis as described in Example 5A. As shown in Figure 12A, anti-IL9 treatment (Asp+ α-mIL9) was able to significantly suppress BHR to levels near that of naïve, while isotype control Ig (Asp + Ig) had no effect on reducing BHR. A similar result was found for airway eosinophilia where a significant eosinophilia resulted upon antigen treatment (Asp -) that was suppressed by anti-mIL9 treatment (Asp + α-mIL9). Northern blot analysis of whole lungs from these mice showed that anti-mIL9 also suppressed ICACC-1 gene expression found in lungs of antigen exposed mice (Figure 13). GAPDH which is a ubiquitously expressed house keeping gene was used as a control to assure equal loading of RNA and overall gene expression. Together, these data demonstrate a tight correlation of ICACC-1 gene expression and the asthmatic response. These data suggest that blocking ICACC-1 expression or function would suppress the asthmatic response.

Example 6: *ICACC-1 inducibility by IL-9 in human lung epithelial cells.*

To assess the ability of ICACC-1 to be induced by IL-9 in epithelial cells, the human

primary lung epithelial cell line NHBE was assayed for expression levels of ICACC-1 in the presence of IL-9. 1×10^7 cells were harvested and washed three times with phosphate-buffered saline and plated in medium in the presence or absence of 50 ng/ml IL-9 for 72 hours. Cells were then harvested and total RNA was extracted using Trizol as described by the manufacturer (Gibco/BRL). RNA was processed and reverse transcribed into cDNA as described in Example 3. Primers used to generate human ICACC-1 message were; sense 5'-GATTCCAGGAACAGCTAAGC-3' (SEQ ID NO:9) and antisense 5'-TATTTTCATAGCTTGTAGCCTGG-3' (SEQ ID NO:10) which produce a gene product of 722 bp. γ -actin was assayed as an internal control to measure for cDNA integrity using primers previously described (Nicolaidis *et al.*, 1991). RT-PCR data derived from human lung epithelial cells, shows that ICACC-1 is induced in cells treated with IL-9 while no expression was observed in untreated cells, indicating that the cells expressing ICACC-1 directly respond to IL-9 (Figure 14).

Furthermore, human primary lung cultures that were established from human lung biopsies were analyzed for IL-9 induced expression of ICACC-1. Lung tissues were first minced with scissors and passed through a wire mesh. Tissues were then digested with 175 iU/ml of collagenase (Sigma) for 1 hour at 37°C. Tissue was passed through 45 μ m and 15 μ m filters and then resuspended in Dulbecco Iscove's medium, and plated into 10 cm tissue culture plates. Plates were incubated for 1 hour at 37°C to allow macrophages to adhere to the plate and then non-adherent cells were harvested and resuspended at 2×10^5 cell/ml in Dulbecco Iscove's medium supplemented with 10% FBS, antibiotics and cultured at 37°C with 5% CO₂ for 4-5 days. For ICACC-1 IL-9 induction studies, cells were incubated for 4-5 days with or without 20 ng/ml recombinant human IL-9. Cells were then harvested and total RNA was extracted by trizol as described above. RNA was reverse transcribed and PCR'd for ICACC-1 using 5' primer 5'-CCCAAAGGAAGCCAAGTCTGA-3' and 3' primer 5'-GTGAATGCCAGGAATGGTGCT-3' which resulted in a 253 bp product. PMS2 which is a ubiquitously expressed house keeping gene was used as an internal control as described (Nicolaidis *et al.* Genomics 29: 329-334, 1995). Products were electrophoresed on 2% agarose gels and visualized by ethidium bromide staining. As shown in Figure 15, IL-9 induced ICACC-1 expression in human primary lung cultures, while cultures grown in the absence of IL-9 had no detectable amounts of ICACC-1.

Example 6A: *ICACC-1 antiserum*

Antisera were prepared to mICACC-1 by immunizing rabbits with peptides selected from the mICACC-1 sequence using methods described in Current Protocols in Immunology, Chapter 9, John Wiley & Sons, Inc. The peptides used for the immunizations were; residues 309-330, CLVLDKSGSMLNDDRLNRMNQA (SEQ ID NO: 11), residues 357-375, QSELKQLNSGADRDLLIKHC (SEQ ID NO: 12), residues 398-422, KKKYPTDGSEIVLLTDGEDNTISSC (SEQ ID NO: 13), residues 524-546, TTHPPTIFTWDPSGVEQNGFILDC (SEQ ID NO: 14), residues 590-610, CPPITVTPVVNKNTGKFSPVT (SEQ ID NO: 15). The peptides were synthesized by standard techniques of automated peptide synthesis as either octavalent multiple antigen peptides (MAP) or as single peptides. The single peptides were coupled to KLH for immunization while the MAPs were used uncoupled. Rabbits were immunized with a mixture of all five peptides either as KLH conjugates or MAPs. Both immunogens produced useful antisera as indicated by their ability to immunoprecipitate mICACC-1.

Immunoprecipitation of in vitro translated ICACC-1 was performed to analyze the activities of these antibodies. A ³⁵S labeled ICACC-1 fragment (429 amino acids in length corresponding to amino acids 289-618 of the full length ICACC-1) was in vitro translated using TNT Coupled Reticulocyte Lysate Systems (Promega). Radio labeled ICACC-1 could be immunoprecipitated by 5 µl of ICACC-1 antisera or 1 µg of protein A purified polyclonal antibody. To assess the specificity of the ICACC-1 antisera, the ³⁵S-labeled mIL-9 receptor fragment (60 KD mIL-9R) was used as a negative control. Under the same precipitation conditions, none of the mIL-9R protein was precipitated by the ICACC-1 antisera (Figure 16). These results indicated that antisera and protein A purified polyclonal antibodies raised against ICACC-1 could recognize ICACC-1 and therefore could potentially be used as pharmaceutical reagents to block ICACC-1 function.

Example 6B: *Suppression of IL-9 induced eotaxin expression in the lung using chloride channel blockers*

IL-9 is known to induce eotaxin from lung epithelial cells (Dong, et.al., submitted for publication, Eur. J. Immunol.). In situ expression analysis of IL-9 transgenic mice found

ICACC-1 expression to be predominant in airway epithelial cells. These epithelial cells also produce eotaxin, and eotaxin can be induced by IL-9 in these cells as well as primary lung cultures from a variety of different mouse strains. Because eotaxin and ICACC-1 are both induced by IL-9 in lung epithelial cells, it is possible that inhibiting ICACC-1 can inhibit eotaxin or other cytokines such as IL-4 or IL-13 (Doucet *et al.*, J. Clin. Invest. 101:2129-2139, 1998.) that induce eotaxin production in lung epithelial cells. To test this hypothesis, we employed a murine primary lung assays, where lung cells were harvested from FVB/NJ mice as described in Example 5A and processed for in vitro analysis as described in Example 6 for human primary lung culture. Cells were incubated with or without 20 ng/ml recombinant mIL-9 for 48 hours. After 48 hours, conditioned supernatant was collected and analyzed for murine eotaxin production using an eotaxin ELISA kit (R&D Systems). Recombinant murine eotaxin was used to generate a standard curve. As shown in Figure 17, FVB primary cells when cultured with IL-9 produce up to 2 ng/ml of eotaxin in contrast to nearly undetectable levels in FVB cultures grown in medium alone. A culture derived from IL-9 transgenic mouse lung (TG5 lane) was used as a positive control. This assay was used to assess the ability to suppress IL-9 induced eotaxin using chloride channel inhibitors DIDS and SITS. Cultures were plated with or without mIL-9 in the presence of 0, 30 μ M and 100 μ M channel blocker. As shown in Figure 18, eotaxin production was inhibited 33% and 41% by 100 μ M DIDS or SITS respectively. These data demonstrate the ability to suppress the biological function of IL-9 on epithelial cells by inhibiting chloride channel function. These data further indicate that suppression of a chloride channel such as the asthma associated ICACC-1 can result in a therapeutic benefit by the suppression of antigen induced asthmatic responses. This screening assay and technique can be used to evaluate other IL-9 induced genes whose products are secreted proteins and is not restricted to using eotaxin as the only marker. A similar approach will be taken using the human ICACC-1 and human functional assays to identify "specific" chloride channel inhibitors that suppress: 1) IL-9 induced effects such as de novo gene expression, and 2) ICACC-1 biologic function(s).

Example 7: Specific blocking of ICACC-1 signaling in vivo by small molecule inhibitors.

To demonstrate the specificity of ICACC-1 signaling which is induced by IL-9,

transfected cells expressing constitutively active ICACC-1 are treated with chloride channel blocking compounds to determine if inhibition of ICACC-1 blocks chloride channel activity. Cells transfected with a constitutively activated ICACC-1 gene are plated at 3000 cells/well in the presence or absence of IL-9 plus blocking compound and assessed for chloride channel activity using a fluorescent chloride probe. Wild-type cells do not exhibit the same amount of chloride channel activity as those constitutively expressing ICACC-1. The addition of the blocking compound on chloride channel activity is compared between wild-type cells and those expressing constitutively activated ICACC-1.

Example 8: Blocking of ICACC-1 induction by aminosterols in murine lung.

Lungs from the DBA bronchial hyperresponsive mouse are treated with aminosterol compounds to test for their ability to block expression of ICACC-1. This group of aminosterols was identified from the liver of the dogfish shark as a class of molecules that appear to be antiproliferative. An example of these compounds are referred to in related U.S. Patent Application Serial No. 08/290,826. This series of aminosterols are assayed for their ability to inhibit ICACC-1 expression and TH2 activity from the DBA mouse as described below.

DBA mice are injected daily intraperitoneally with various aminosterols at 10 mg/kg for 15 days. At day 15, mice are phenotyped (see Example 9), euthanized and lungs extracted as described in Example 1. RNA is isolated and processed for Northern blot analysis using a ICACC-1 cDNA probe. The level of ICACC-1 RNA detected by the probe indicates the extent of inhibition by aminosterols when compared to control. The ability of specific aminosterols, such as 1459, 1409, 1436 and 1569, to block the expression of ICACC-1 in vivo is assessed.

Example 9: Role of ICACC-1 in murine models of asthma: Airway response of unsensitized animals.

Certified virus-free male and female mice of the following strains, DBA, C57B6 and B6D2F1 are purchased from the National Cancer Institute or Jackson Laboratories (Bar Harbor ME). IL-9 transgenic mice (Tg5) and their parent strain (FVB), are obtained from the Ludwig Institute (Brussels, Belgium). Animals are housed in high-efficiency particulate

filtered air laminar flow hoods in a virus and antigen free facility and allowed free access to food and water for 3 to 7 days prior to experimental manipulation. The animal facilities are maintained at 22°C and the light:dark cycle is automatically controlled (10:14 hour light:dark).

- 5 *Phenotyping and efficacy of pretreatment.* To determine the bronchoconstrictor response, respiratory system pressure is measured at the trachea and recorded before and during exposure to the drug. Mice are anesthetized and instrumented as previously described. (Levitt *et al.*, 1988; Levitt *et al.*, 1989; Kleeberger *et al.*, 1990; Levitt *et al.*, 1991; Levitt *et al.*, 1995; Ewart *et al.*, 1995). Airway responsiveness is measured to one or more of the
- 10 following: 5-hydroxytryptamine, acetylcholine, atracurium or a substance-P analog. A simple and repeatable measure of the change in peak inspiratory pressure following bronchoconstrictor challenge is used which has been termed the Airway Pressure Time Index (APTI) (Levitt *et al.*, 1988; Levitt *et al.*, 1989). The APTI is assessed by the change in peak respiratory pressure integrated from the time of injection until the peak pressure returns to
- 15 baseline or plateau. The APTI is comparable to airway resistance, however, the APTI includes an additional component related to the recovery from bronchoconstriction.

Prior to sacrifice, whole blood is collected for serum IgE measurements by needle puncture of the inferior vena cava in anesthetized animals. Samples are centrifuged to separate cells and serum is collected and used to measure total IgE levels. Samples not

20 measured immediately are frozen at -20°C.

All IgE serum samples are measured using an ELISA antibody-sandwich assay. Microtiter plates are coated, 50 µl per well, with rat anti-murine IgE antibody (Southern Biotechnology) at a concentration of 2.5 µg/ml in a coating buffer of sodium carbonate-sodium bicarbonate with sodium azide. Plates are covered with plastic wrap and

25 incubated at 4°C for 16 hours. The plates are washed three times with a wash buffer of 0.05% Tween-20 in phosphate-buffered saline, incubating for five minutes for each wash. Blocking of nonspecific binding sites is accomplished by adding 200 µl per well 5% bovine serum albumin in phosphate-buffered saline, covering with plastic wrap and incubating for 2 hours at 37°C. After washing three times with wash buffer, duplicate 50 µl test samples are

30 added to each well. Test samples are assayed after being diluted 1:10, 1:50 and 1:100 with 5% bovine serum albumin in wash buffer. In addition to the test samples, a set of IgE

standards (PharMingen) at concentrations from 0.8 ng/ml to 200 ng/ml in 5% bovine serum albumin in wash buffer, are assayed to generate a standard curve. A blank of no sample or standard is used to zero the plate reader (background). After adding samples and standards, the plate is covered with plastic wrap and incubated for 2 hours at room temperature. After
5 washing three times with wash buffer, 50 μ l of secondary antibody rat anti-murine IgE-horseradish peroxidase conjugate is added at a concentration of 250 ng/ml in 5% bovine serum albumin in wash buffer. The plate is covered with plastic wrap and incubated 2 hours at room temperature. After washing three times with wash buffer, 100 μ l of the substrate 0.5 mg/ml o-phenylenediamine in 0.1 M citrate buffer is added to every well. After 5-10 minutes
10 the reaction is stopped with 50 μ l of 12.5% sulfuric acid and absorbance is measured at 490 nm on a MR5000 plate reader (Dynatech). A standard curve is constructed from the standard IgE concentrations with antigen concentration on the x axis (log scale) and absorbance on the y axis (linear scale). The concentration of IgE in the samples is interpolated from the standard curve.

15 Bronchoalveolar lavage and cellular analysis are performed as previously described (Kleeberger *et al.*, 1990). Lung histology is carried out after the lungs are extracted. Since prior instrumentation may introduce artifact, separate animals are used for these studies. Thus, a small group of animals is treated in parallel exactly the same as the cohort undergoing various pretreatments except these animals are not used for other tests aside from
20 bronchial responsiveness testing. After bronchial responsiveness testing, the lungs are removed and submersed in liquid nitrogen. Cryosectioning and histologic examination is carried out in a manner obvious to those skilled in the art.

Polyclonal antibodies which block the murine ICACC-1 pathway are used therapeutically to down-regulate the functions of, and assess the importance of this pathway
25 to bronchial responsiveness, serum IgE and bronchoalveolar lavage in sensitized and unsensitized mice. After antibody pretreatment, baseline bronchial hyperresponsiveness, bronchoalveolar lavage and serum IgE levels relative to Ig matched controls are determined.

Example 10: Role of ICACC-1 in murine models of asthma: Airway response of sensitized animals.

30 The data of Example 6a demonstrate that antisera is able to be generated against ICACC-

- 1 that recognizes the native protein structure as determined by the ability to recognize the protein in immunoprecipitation studies (Figure 16). ICACC-1 blocking antibodies represent potential therapeutic agents in suppressing the function of ICACC-1. Studies are carried out using antigen sensitized animals and protocols as described Examples 5A, 5B, and 10.
- 5 Animals are given ICACC-1 blocking antibodies via intranasal administration as described in example 5B and at day 23 animals are phenotyped for BHR, BAL, and immunoglobulin levels. The effect of pretreatment with ICACC-1 antibodies is used to assess the effect of down-regulating ICACC-1 on the asthma phenotype.

While the invention has been described and illustrated herein by references to various
10 specific materials, procedures and examples, it is understood that the invention is not restricted to the particular combinations of material and procedures selected for that purpose. Numerous variations of such details can be implied as will be appreciated by those skilled in the art.

REFERENCES

The following references are herein incorporated by reference in their entirety, as are all references, patents or patent applications referred to in this application:

- Alexander AG, Barnes NC and Kay AB. Trial of cyclosporin in corticosteroid-dependent
5 chronic severe asthma. Lancet 339, 324-328, 1992.
- Altschul SF, Gish W, Miller W, Myers EW and Lipman DJ. Basic Local alignment search
tool. J. Mol. Biol. 215, 403-410, 1990.
- Burrows B, Sears MR, Flannery EM, Herbison GP and Holdaway MD. Relationship of
bronchial responsiveness assessed by methacholine to serum IgE, lung function,
10 symptoms and diagnoses in 11-year-old New Zealand children. J. Allergy Clin.
Immunol. 90, 376-385, 1992.
- Burrows B, Martinez FD, Halonen M, Barbee RA and Cline MG. Association of asthma
with serum IgE levels and skin-test reactivity to allergens. New Eng. J. Med. 320,
271-277, 1989.
- 15 Cotton RG. Detection of single base changes in nucleic acids. Biochemical Journal 263(1),
1-10, 1989.
- Chu JW and Sharom FJ. Glycophorin A interacts with interleukin-2 and inhibits interleukin-
2-dependent T-lymphocyte proliferation. Cell. Immunol. 145, 223-239, 1992.
- Clifford RD, Pugsley A, Radford M and Holgate ST. Symptoms, atopy and bronchial
20 response to methacholine in parents with asthma and their children. Arch. Dis.
Childhood 62, 66-73, 1987.
- Cunningham SA, Awayda MS, Bubien JK, Ismailov II, Arrate MP, Berdiev BK, Benos DJ
and Fuller CM. Cloning of an epithelial chloride channel from bovine trachea. J. Biol.
Chem. 270, 31016-31026, 1995.
- 25 Doull I, Lawrence S, Watson M, Begishvili T, Beasley R, Lampe F, Holgate ST and Morton
NE. Allelic association of makers on chromosome 5q and 11q with atopy and bronchial
hyperresponsiveness. Am. J. Respir. Crit. Care Med. 153, 1280-1284, 1996.
- Dugas B, Renauld JC, Pene J, Bonnefoy J, Peti-Frere C, Braquet P, Bousquet J, Van Snick J,
Mencia-Huerta JM. Interleukin-9 potentiates the interleukin-4-induced immunoglobulin
30 (IgG, IgM and IgE) production by normal human B lymphocytes. Eur. J. Immunol. 23,

1687-1692, 1993.

Eklund KK, Ghildyal N, Austen KF and Stevens RL. Induction by IL-9 and suppression by IL-3 and IL-4 of the levels of chromosome 14-derived transcripts that encode late-expressed mouse mast cell proteases. J. Immunol. 151, 4266-4273, 1993.

- 5 Eng PA, Morton J, Douglass JA, Riedler J, Wilson J and Robertson CF. Short-term efficacy of ultrasonically nebulized hypertonic saline in cystic fibrosis. Pediatr Pulmonol. 21, 77-83, 1996.

Ewart S, Levitt RC and Mitzner W. Respiratory system mechanics in mice measured by end-inflation occlusion. J. Appl. Phys. 79, 560-566, 1995.

- 10 Gergen PJ and Weiss KB. The increasing problem of asthma in the United States. Am. Rev. Respir. Dis. 146, 823-824, 1992.

Gergen PJ. The association of allergen skin test reactivity and respiratory disease among whites in the U.S. population. Arch. Intern. Med. 151, 487-492, 1991.

- 15 Halonen M, Stern D, Taussig LM, Wright A, Ray CG and Martinez FD. The predictive relationship between serum IgE levels at birth and subsequent incidences of lower respiratory illnesses and eczema in infants. Am. Rev. Respir. Dis. 146, 666-670, 1992.

Johannsson SGO, Bennich HH and Berg T. The clinical significance of IgE. Prog. Clin. Immunol. 1, 1-25, 1972.

- 20 Johnson LG, Boyles SE, Wilson J and Boucher RC. Normalization of raised sodium absorption and raised calcium-mediated chloride secretion by adenovirus-mediated expression of cystic fibrosis transmembrane conductance regulator in primary human cystic fibrosis airway epithelial cells. J Clin Invest. 95, 1377-1382, 1995.

Kauvar LM. Peptide mimetic drugs: A comment on progress and prospects. Nature Biotechnology 14, 709, 1996.

- 25 Kleeberger SR, Bassett DJ, Jakab GJ and Levitt RC. A genetic model for evaluation of susceptibility to ozone-induced inflammation. Am. J. Physiol. 258, L313-320, 1990.

Kreitman RJ, Puri RK, Leland P *et al.* Site-specific conjugation to interleukin-4 containing mutated cysteine residues produces interleukin 4-toxin conjugates with improved binding and activity. Biochemistry 33, 11637-11644, 1994.

- 30 Levitt RC and Ewart SL. Genetic susceptibility to atracurium-induced bronchoconstriction. Am. J. Respir. Crit. Care. Med. 151, 1537-1542, 1995.

- Levitt RC. Understanding biological variability in susceptibility to respiratory disease. Pharmacogenetics 1, 94-97, 1991.
- Levitt RC and Mitzner W. Autosomal recessive inheritance of airway hyperreactivity to 5-hydroxytryptamine. J. Appl. Physiol. 67, 1125-1132, 1989.
- 5 Levitt RC, Mitzner W *et al.* Expression of airway hyperreactivity to acetylcholine as a simple autosomal recessive trait in mice. FASEB J. 2, 2605-2608, 1988.
- Louahed J, Kermouni A, Van Snick J and Renaud JC. IL-9 induces expression of granzymes and high affinity IgE receptor in murine T helper clones. J. Immunol. 154, 5061-5070, 1995.
- 10 Marsh DG, Meyers DA and Bias WB. The epidemiology and genetics of atopic allergy. New Eng. J. Med. 305, 1551-1559, 1982.
- Molinoff P *et al.*, Goodman and Gilman's The Pharmacologic Basis of Therapeutics, MacMillan Publishing Company, New York NY, 1995.
- Morely J. Cyclosporin A in asthma therapy: A pharmacological rationale. J. Autoimmun. 5
15 Suppl A, 265-269, 1992.
- Nicolaides *et al.* IL-9: A candidate gene for asthma. Proc. Natl. Acad. Sci. USA 94, 13175-13180, 1997.
- Nicolaides NC, Carter KC, Shell BK, Papadopoulos N, Vogelstein B and Kinzler KW. Genomic organization of the human PMS2 gene family. Genomics 30, 195-206, 1995.
- 20 Nicolaides NC, Gualdi R, Casadevall C, Manzella L, Calabretta B *et al.* Positive autoregulation of c-myb expression via Myb binding sites in the 5' flanking region of the human c-myb gene. Mol. Cell. Biol. 11, 6166-6176, 1991.
- Orita M, Suzuki Y, Sekiya T and Hayashi K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5,
25 874-879, 1989.
- Ott J. Analysis of human genetic linkage. John Hopkins University Press, Baltimore MD, 1991.
- Petit-Frere C, Dugas B, Braquet P, Mencia-Huerta JM. Interleukin-9 potentiates the interleukin-4-induced IgE and IgG1 release from murine B lymphocytes. Immunology
30 79, 146-151, 1993.
- Renaud JC, van der Lugt N, Vink A, van Roon M, Godfraind C, Warnier G, Merz H, Feller

- A, Berns A and Van Snick J. Thymic lymphomas in interleukin 9 transgenic mice. Oncogene 9, 1327-1332, 1994.
- Sarkar G, Yoon H-S and Sommer SS. Dideoxy fingerprint (ddF): A rapid and efficient screen for the presence of mutations. Genomics 13, 441-443, 1992.
- 5 Schwengel D, Nouri N, Meyers D and Levitt RC. Linkage mapping of the human thromboxane A2 receptor (TBXA2R) to chromosome 19p13.3 using transcribed 3' untranslated DNA sequence polymorphisms. Genomics 18, 212-215, 1993.
- Sears MR, Burrows B, Flannery EM, Herbison GP, Hewitt CJ and Holdaway MD. Relation between airway responsiveness and serum IgE in children with asthma and in apparently
10 normal children New Engl. J. Med. 325(15), 1067-1071, 1991.
- Sheffield VC, Beck JS, Kwitek AE, Sandstrom DW and Stone EM. The sensitivity of single-strand conformation polymorphism analysis for the detection of single base substitutions. Genomics 16, 325-332, 1993.
- Simoncsits A, Bristulf J, Tjornhammar ML *et al.* Deletion mutants of human interleukin 1
15 beta significantly reduced agonist properties: search for the agonist/antagonist switch in ligands to the interleukin 1 receptors. Cytokine 6, 206-214, 1994.
- Zavjalov VP, Navolotskaya EV, Isaev IS *et al.* Nonapeptide corresponding to the sequence 27-35 of the mature human IL-2 efficiently competes with rIL-2 for binding to thymocyte receptors. Immunol. Lett. 31, 285-288, 1992.

What Is Claimed:

1. A purified and isolated nucleic acid molecule selected from the group consisting of a nucleic acid molecule having a nucleotide sequence encoding human ICACC-1 (SEQ ID NO:6), a nucleic acid molecule which hybridizes to a nucleic acid molecule having SEQ ID NO: 1 and a nucleic acid molecule comprising functionally effective fragments thereof.
2. A purified and isolated DNA molecule having a nucleotide sequence encoding murine ICACC-1 (SEQ ID NO:2) or functionally effective fragments thereof.
3. A purified and isolated DNA molecule having a nucleotide sequence encoding human ICACC-2 (SEQ ID NO:4) or functionally effective fragments thereof.
4. The purified and isolated DNA molecule of claim 1, 2 or 3 wherein said DNA molecule is genomic.
5. A chemically synthesized DNA molecule having a nucleotide sequence encoding human ICACC-1 or functionally effective fragments thereof.
6. A chemically synthesized DNA molecule having a nucleotide sequence encoding murine ICACC-1 or functionally effective fragments thereof.
7. A chemically synthesized DNA molecule having a nucleotide sequence encoding human ICACC-2 or functionally effective fragments thereof.
8. A purified and isolated RNA molecule having a nucleotide sequence encoding human ICACC-1 or functionally effective fragments thereof.
9. A purified and isolated RNA molecule having a nucleotide sequence encoding murine ICACC-1 or functionally effective fragments thereof.
10. A purified and isolated RNA molecule having a nucleotide sequence encoding human ICACC-2 or functionally effective fragments thereof.
11. A purified and isolated protein molecule having an amino acid sequence comprising human ICACC-1 (SEQ ID NO:6) or functionally effective fragments thereof.
12. A purified and isolated protein molecule having an amino acid sequence comprising murine ICACC-1 (SEQ ID NO:2) or functionally effective fragments thereof.
13. A purified and isolated protein molecule having an amino acid sequence comprising human ICACC-1 (SEQ ID NO:4) or functionally effective fragments thereof.
14. A method of alleviating asthma by administering to patients in need of such treatment an effective amount of a compound to down-regulate the function of human ICACC-1.

15. A method according to claim 14 wherein the compound is a chloride channel inhibitor.
16. A method according to claim 14 wherein the compound is an aminosterol.
17. A method of alleviating asthma by administering to patients in need of such treatment an effective amount of an antibody that blocks the function of an ICACC thereby down-
 - 5 regulating the activity of an ICACC-1.
 18. The method of claim 17 wherein the antibody is produced from peptides that encompass the functional domains of ICACC-1.
 19. A method according to claim 17 wherein the antibody is produced from peptides taken from the group of SEQ ID NO: 11-15.
- 10 20. The method of claim 17 wherein the antibody is monoclonal.
21. A method for detecting or diagnosing susceptibility to asthma associated with elevated levels of an ICACC-1 polypeptide in a human subject comprising the steps of:
 - (a) measuring the level of the ICACC-1 polypeptide in a biological sample from said human subject; and
 - 15 (b) comparing the level of ICACC-1 polypeptide present in normal subjects, wherein an increase in the level of ICACC-1 polypeptide as compared to normal levels indicates a predisposition to asthma.
22. A method for monitoring a therapeutic treatment of asthma associated with elevated levels of an ICACC-1 polypeptide in a human subject comprising; measuring the levels of
 - 20 the ICACC-1 polypeptide in a series of biologic samples obtained at different time points from said subject undergoing therapeutic treatment wherein a significant decrease in said levels of ICACC-1 polypeptide indicates a successful therapeutic treatment.
23. A method of preparing an antibody specific to an ICACC polypeptide which comprises the protein molecule of claims 11 or 12 or fragments thereof comprising the steps of:
 - 25 (a) conjugating an ICACC polypeptide which comprises SEQ ID NO:6 or SEQ ID NO:4 or fragments thereof containing at least ten amino acids to a carrier protein;
 - (b) immunizing a host animal with said ICACC polypeptide fragment-carrier protein conjugate admixed with an adjuvant; and
 - (c) obtaining antibody from the immunized host animal.
- 30 24. A method according to claim 23 wherein the protein fragment is taken from the group of SEQ ID NO: 11-15.

25. A method of quantifying an ICACC polypeptide of claims 11 or 12 comprising the steps of:

- (a) contacting a sample suspected of containing the ICACC polypeptide with an antibody that specifically binds to the ICACC polypeptide under conditions that allow for the formation of reaction complexes comprising the antibody and the ICACC polypeptides; and
- (b) detecting the formation of reaction complexes comprising the antibody and the ICACC polypeptide in the sample, wherein quantitation of the reaction complexes indicates the level of the ICACC polypeptide in the sample.

26. A method for identifying antagonists of an ICACC-1 comprising the steps of:

- (a) obtaining non-human animals susceptible to airway hyperresponsiveness;
- (b) administering antigens that induce airway hyperresponsiveness;
- (c) comparing the characteristics of any resulting airway hyperresponsiveness with the characteristics of airway hyperresponsiveness obtained with pretreatment with a possible ICACC-1 antagonist agent; and
- (d) selecting those agents for which pretreatment diminished the characteristics.

27. The method of claim 26 wherein the animal expresses human ICACC-1.

28. A method for identifying antagonists of an ICACC-1 comprising the steps of:

- (a) obtaining a cell line that is responsive to IL-9;
- (b) growing said cell line in the presence of IL-9;
- (c) comparing the characteristics of IL-9 induction with those obtained with pretreatment with a possible ICACC-1 antagonist agent; and
- (d) selecting those agents for which pretreatment diminished the characteristics.

29. The method according to claim 27 wherein the cell line is: murine lung epithelial cells transfected with the human IL-9 receptor or human NHBE cells.

30. Antisense DNA comprising the antisense sequence of a human ICACC-1 or active fragments thereof.

31. A method according to claim 14 wherein the compound is the antisense DNA of claim 30.

32. A method of treating cystic fibrosis by administering to patients in need of such treatment an effective amount of a compound to up-regulate the function of a human ICACC.

33. A method of treating inflammatory bowel disease by administering to patients in need of such treatment an effective amount of a compound to up-regulate the function of a human ICACC-1.
34. A method according to claims 32 or 33 wherein the compound is IL-9.
- 5 35. A method for detecting or diagnosing susceptibility to inflammatory bowel disease associated with reduced levels of an ICACC-1 polypeptide in a human subject comprising the steps of:
- (a) measuring the level of ICACC-1 polypeptide in a biological sample from said human subject; and
 - 10 (b) comparing the level of ICACC-1 polypeptide present in normal subjects, wherein an decrease in the level of ICACC-1 polypeptide as compared to normal levels indicates a predisposition to inflammatory bowel disease.
36. A method for monitoring a therapeutic treatment of inflammatory bowel disease associated with reduced levels of an ICACC-1 polypeptide in a human subject comprising;
- 15 measuring the levels of ICACC-1 polypeptide in a series of biologic samples obtained at different time points from said subject undergoing therapeutic treatment wherein a significant increase in said levels of ICACC-1 polypeptide indicates a successful therapeutic treatment.

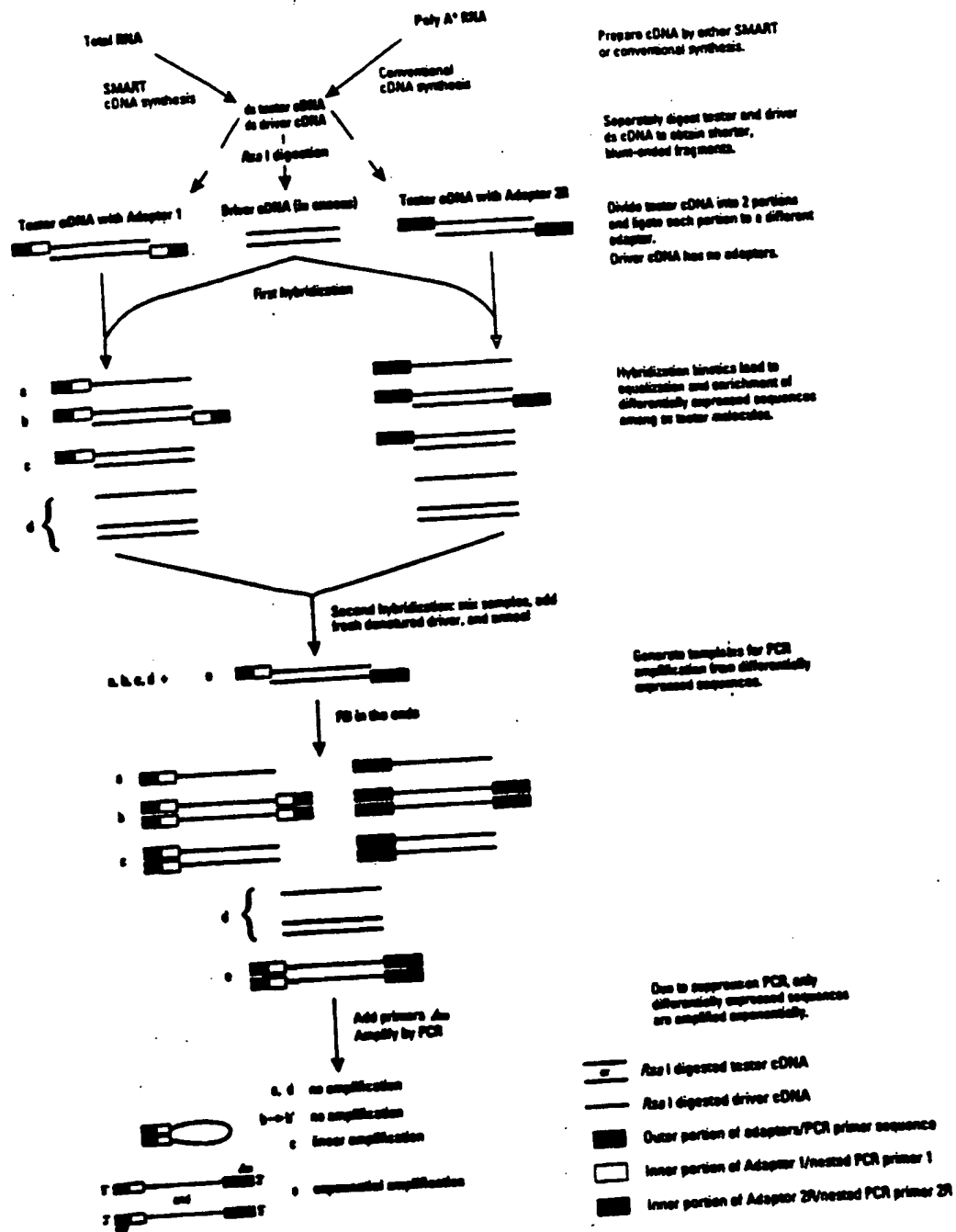


FIG. 1

FIG. 3 mCACC-1 Aligned Formatted Alignments

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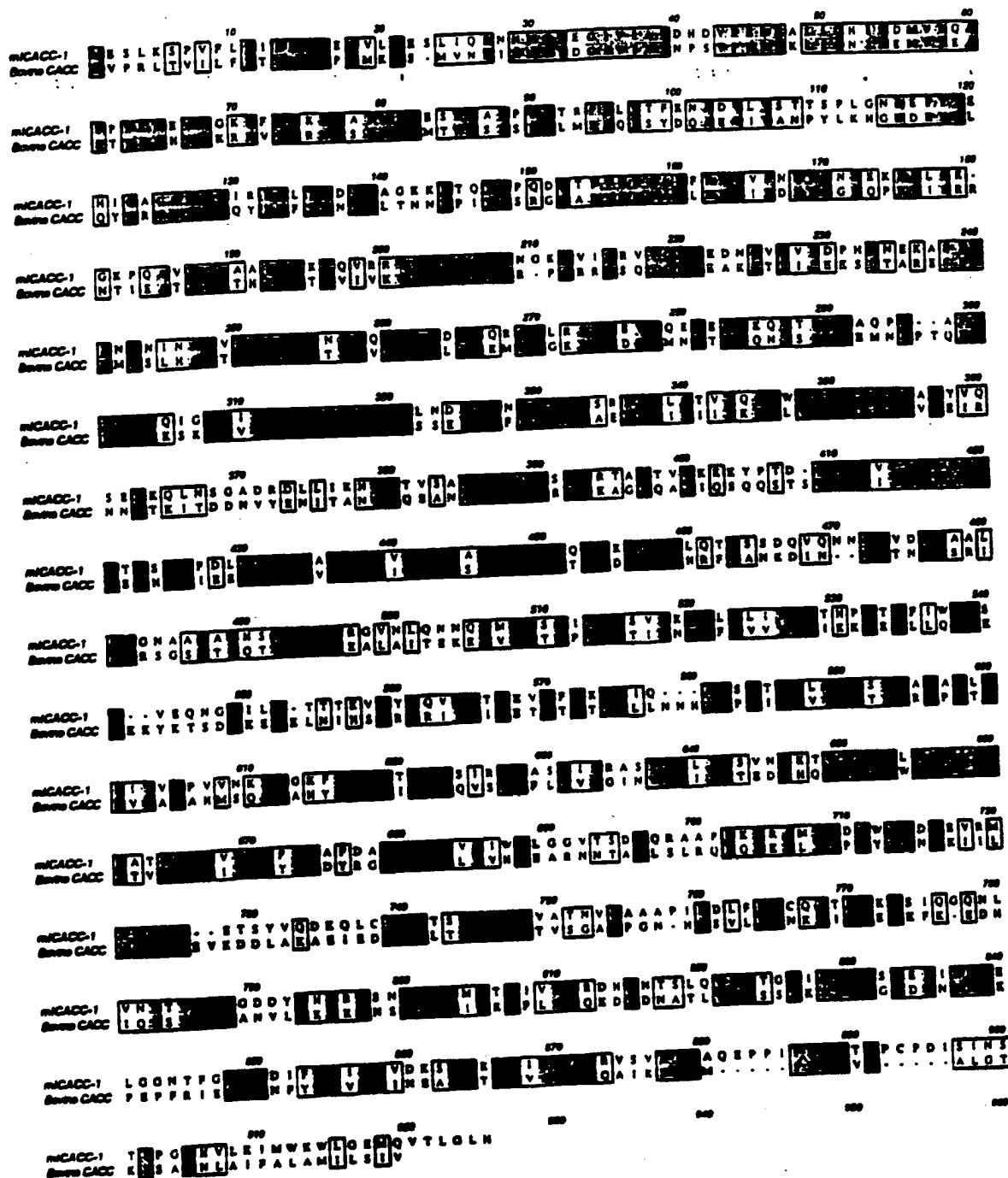


FIG. 3

SEQUENCE-1 Translated sequence

Sequence Range: 1 to 3199

10 20 30 40 50 60 70 80 90 100 110 120
 CTT GTT GTT TTT TTA AAC CTT TCC AAG TTC AGC AAA AAA GGC ATC TCC ATC CAT ATT GAA AAC CTG ACA CAA TGT ATG CAG CAG GCT CAG TGT GAG TGA ACT GGA GGC TTC TCT GGA ACA
 130 140 150 160 170 180 190 200 210 220 230 240
 TGA GCG AAA GGA GCA TTA CAG GTC CTA TTT GCA ACC TTA ATG TTA TTA CTC TGG TGG CTT TAA CTT CAG AAC TCC CAT TCC TGG GAG GTC GAG TAC ACC TTC AAG ACA ATG GGT ATA
 250 260 270 280 290 300 310 320 330 340 350 360
 ATG GGT TCC TCA TTA CAA TTA ATC CTC AAG TAC CTG GAG AAA ACC AAA ACC TCA TCT CAA ACA TTA AAA TAA TAA CTG AAG CTT CTT TTT ACC TGT TTA CTG CTA CCA AAA GAA GAG TAT
 370 380 390 400 410 420 430 440 450 460 470 480
 TTT TCA GAA AAA TAA AAA TTT TAA TAT GTC GCA CAG GAA AAA TTA AAA ACA GCA AAA TAA AAC AAA CAG CAG AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA
 490 500 510 520 530 540 550 560 570 580 590 600
 GAG AAA ATC CAG ACA CTT TAC AAT ACA GAG GGT GAA AAA
 610 620 630 640 650 660 670 680 690 700 710 720
 TGT TTA TCC AAA AAT GAG GGC ACC TCC GGT GAG GGT TTT TTA AAA
 730 740 750 760 770 780 790 800 810 820 830 840
 CAG GCA TTT
 850 860 870 880 890 900 910 920 930 940 950 960
 TTT TCA TTT GAA GGT TTT
 970 980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080
 AAT GTC GTC AAT TTT ACC ACA GCT TTT GCA TAA AAA GAG GTC AAA TTT
 1090 1100 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
 AAA TTA GAG GTC AAA GAG TTT
 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320
 GAG GTC AAA GAG TTT
 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440
 AAA TTA GAG GTC AAA GAG TTT
 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
 AAT GTC TTA GTC TTT GGT
 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680
 GAG GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT
 1690 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
 GCA AAA GTC TTT
 1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
 CAG GTC GGT TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT
 1930 1940 1950 1960 1970 1980 1990 2000 2010 2020 2030 2040
 CTG GTC GAG GTC TTT
 2050 2060 2070 2080 2090 2100 2110 2120 2130 2140 2150 2160
 CTG GTC CAG TTT AAA GTC GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT GTC TTT
 2170 2180 2190 2200 2210 2220 2230 2240 2250 2260 2270 2280
 CAA AAA GTC GGT GTC TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA
 2290 2300 2310 2320 2330 2340 2350 2360 2370 2380 2390 2400
 TTT AAA TTT AAA GTC CAA GAA AAT CAG TTT GTC AAA
 2410 2420 2430 2440 2450 2460 2470 2480 2490 2500 2510 2520
 CTG AAA TTT TTT GTC CAG GTC AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT
 2530 2540 2550 2560 2570 2580 2590 2600 2610 2620 2630 2640
 AAA TTA GTC TTT GTC AAA GTC TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA
 2650 2660 2670 2680 2690 2700 2710 2720 2730 2740 2750 2760
 GTC AAA TTT GTC GTC AAA GTC TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA
 2770 2780 2790 2800 2810 2820 2830 2840 2850 2860 2870 2880
 TTT AAA GTC GTC TTT
 2890 2900 2910 2920 2930 2940 2950 2960 2970 2980 2990 3000
 TTT TTT GTC AAA GTC TTT GTC AAA GTC TTT GTC AAA GTC TTT GTC AAA GTC TTT GTC AAA GTC TTT GTC AAA GTC TTT GTC AAA GTC TTT GTC AAA GTC TTT GTC AAA GTC
 3010 3020 3030 3040 3050 3060 3070 3080 3090 3100 3110 3120
 GAG TTA AAA AAC AAA GTC ACA AAT TTA AAA GAG CAG AAA TTT AAA
 3130 3140 3150 3160 3170 3180 3190
 GAG TTA AAA ACC TTA CAG TTT GTC TTT GTC AAA

FIG. 4A

FIGURE 1

Sequence Range: 1 to 2745

10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350 360 370 380 390 400 410 420 430 440 450 460 470 480 490 500 510 520 530 540 550 560 570 580 590 600 610 620 630 640 650 660 670 680 690 700 710 720 730 740 750 760 770 780 790 800 810 820 830 840 850 860 870 880 890 900 910 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800 1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920 1930 1940 1950 1960 1970 1980 1990 2000 2010 2020 2030 2040 2050 2060 2070 2080 2090 2100 2110 2120 2130 2140 2150 2160 2170 2180 2190 2200 2210 2220 2230 2240 2250 2260 2270 2280 2290 2300 2310 2320 2330 2340 2350 2360 2370 2380 2390 2400 2410 2420 2430 2440 2450 2460 2470 2480 2490 2500 2510 2520 2530 2540 2550 2560 2570 2580 2590 2600 2610 2620 2630 2640 2650 2660 2670 2680 2690 2700 2710 2720 2730 2740 2745

FIG. 4B

ClustalW Formatted Alignments

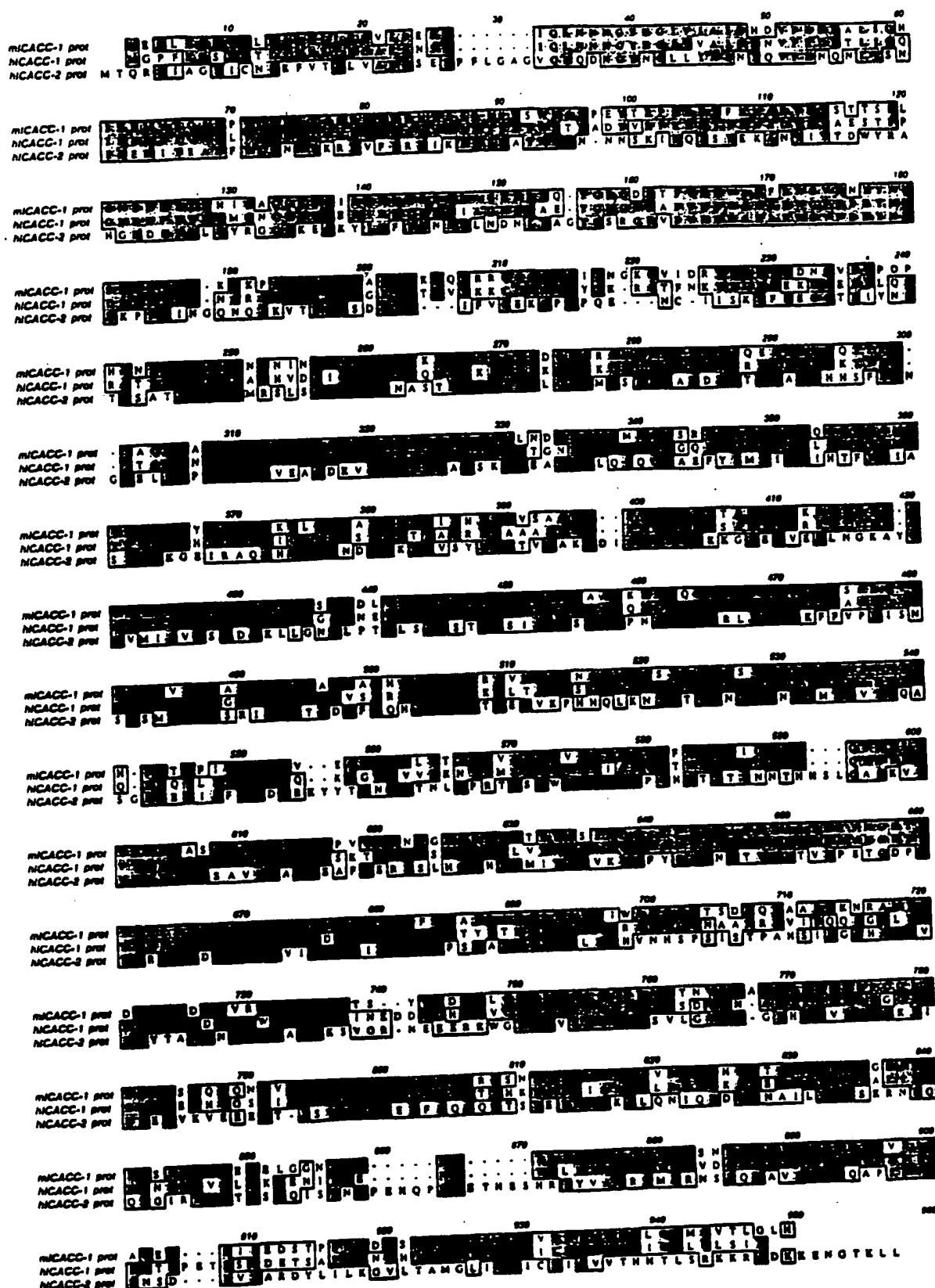


FIG. 5

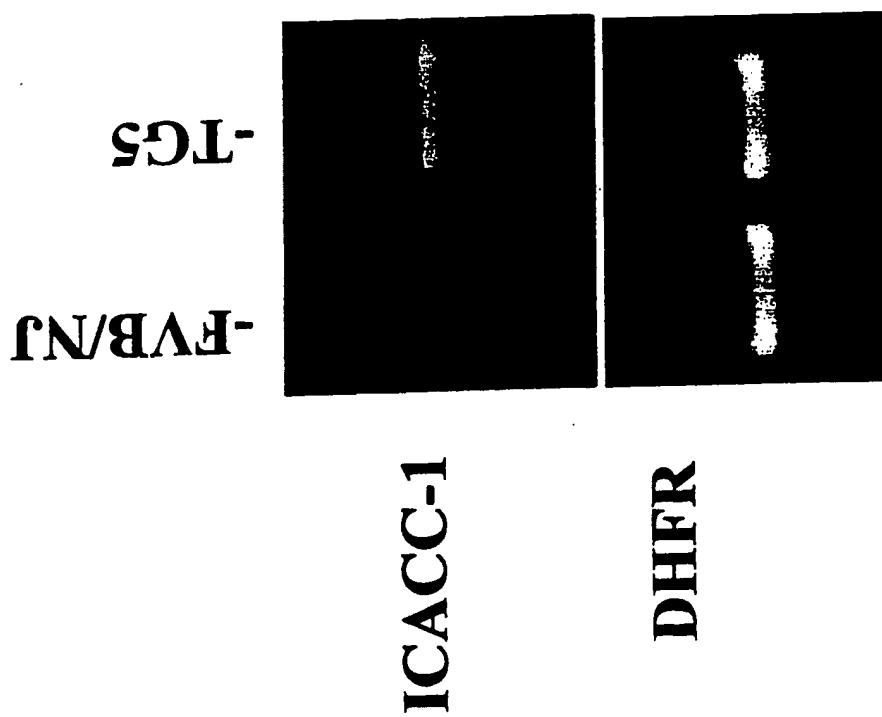


Fig 6

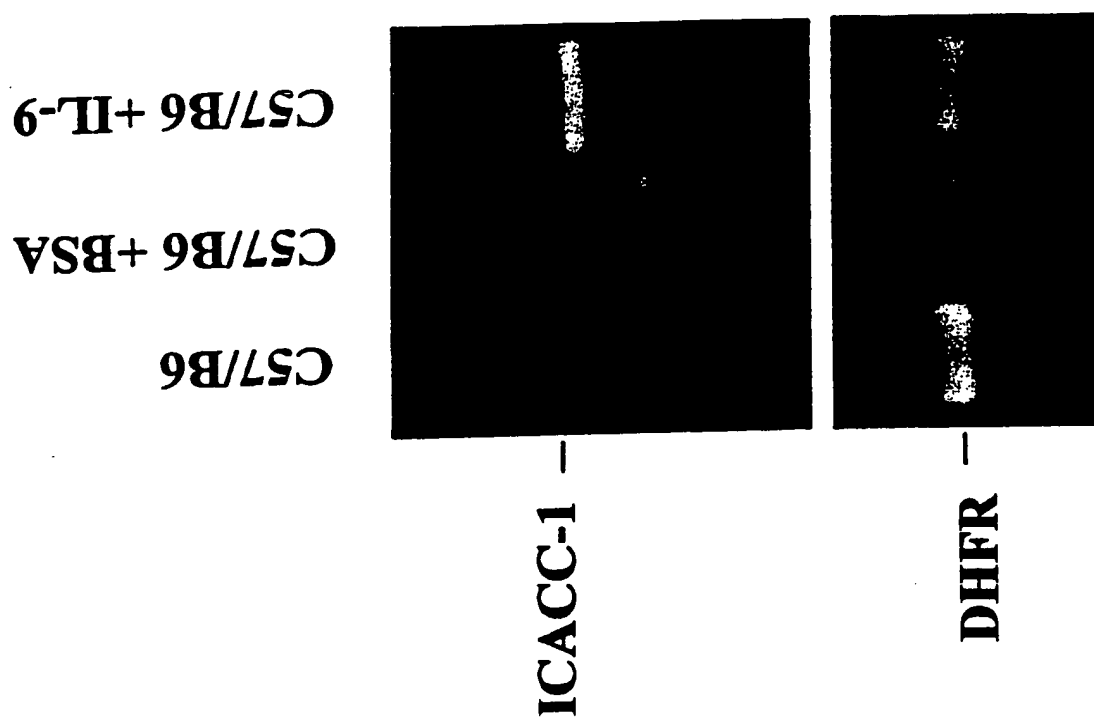
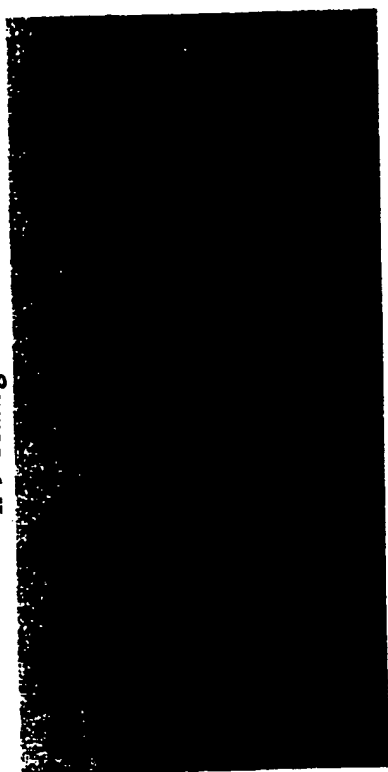


Fig 8

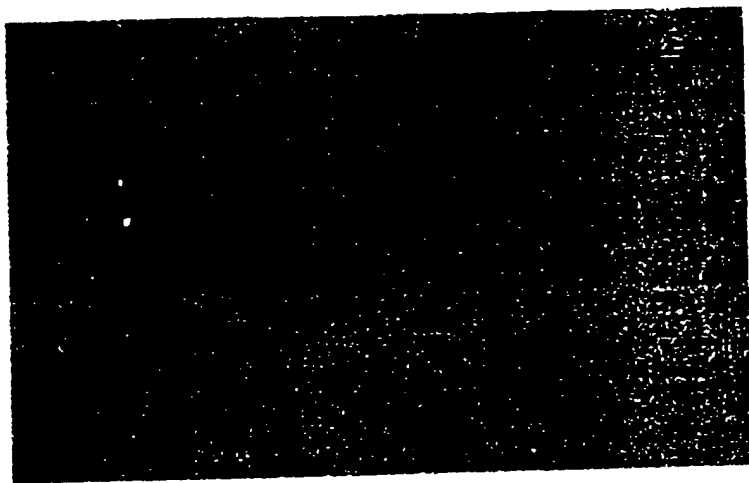
11-9 Transgenic Mouse

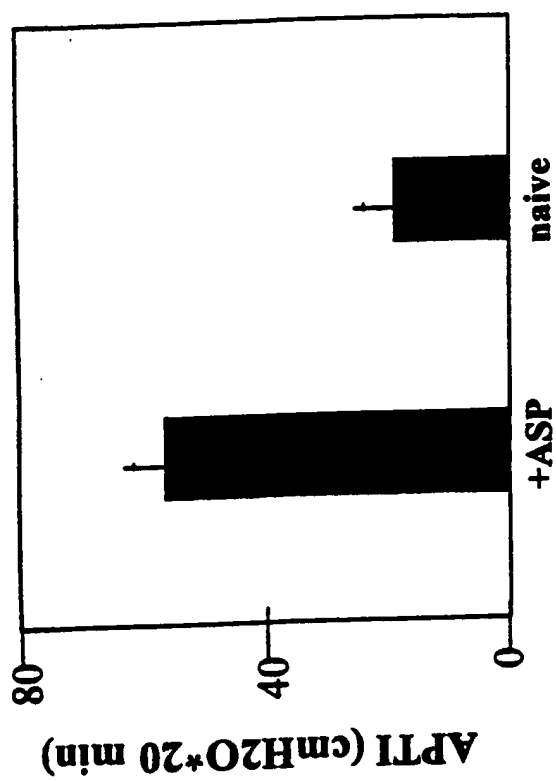
1. uterus
2. thymus
3. testis
4. stomach
5. spleen
6. smt. intestine
7. ovary
8. muscle
9. lymph nodes
10. lung
11. liver
12. kidney
13. heart
14. colon
15. brain



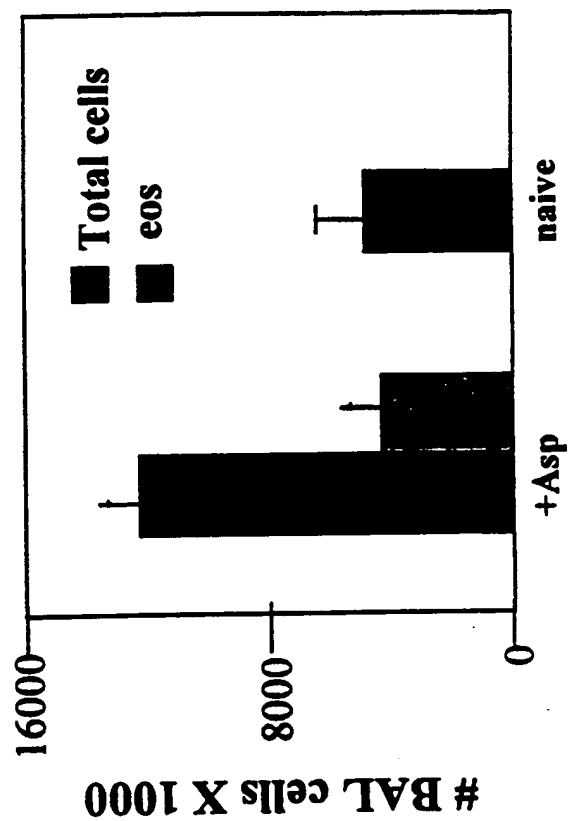
Normal Mouse

1. heart
2. brain
3. spleen
4. lung
5. liver
6. skeletal muscle
7. kidney
8. testis





A



B

Fig 10

1. BM
2. Brain
3. Colon
4. Heart
5. Kidney
6. Liver
7. Lung
8. Lymphnode
9. Muscle
10. Ovary
11. Small Intestine
12. Spleen
13. Stomach
14. Thymus
15. Uterus

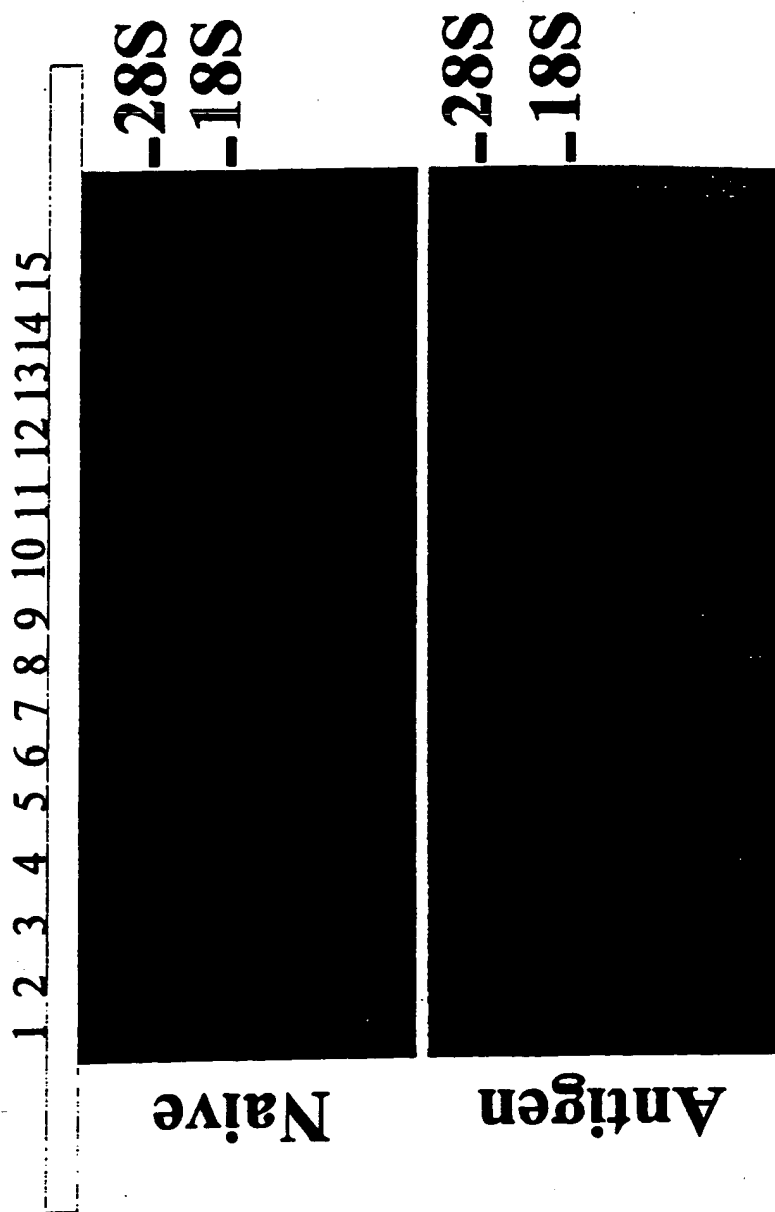


Fig 11

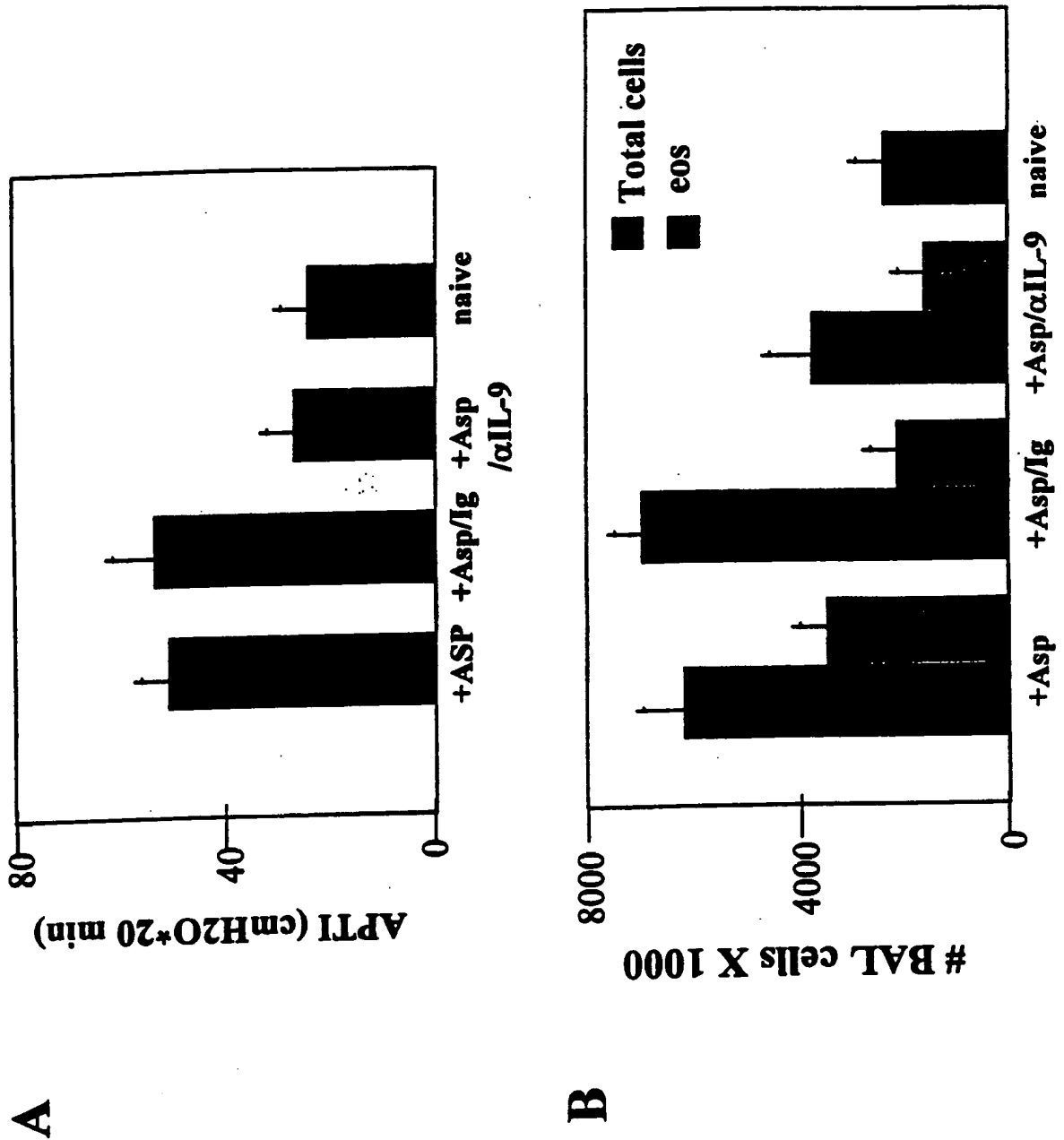


Fig 12

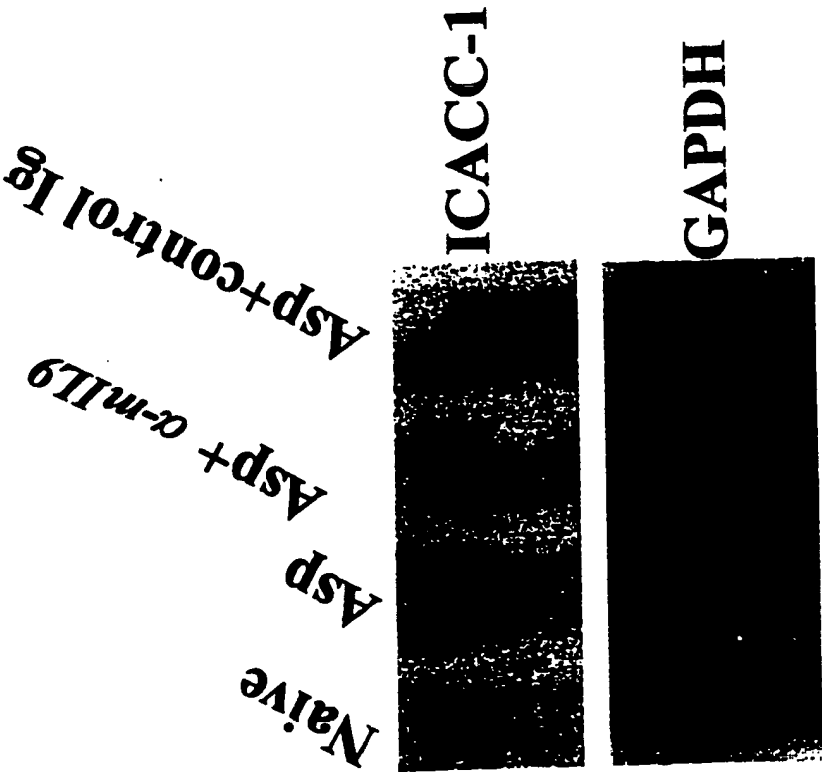
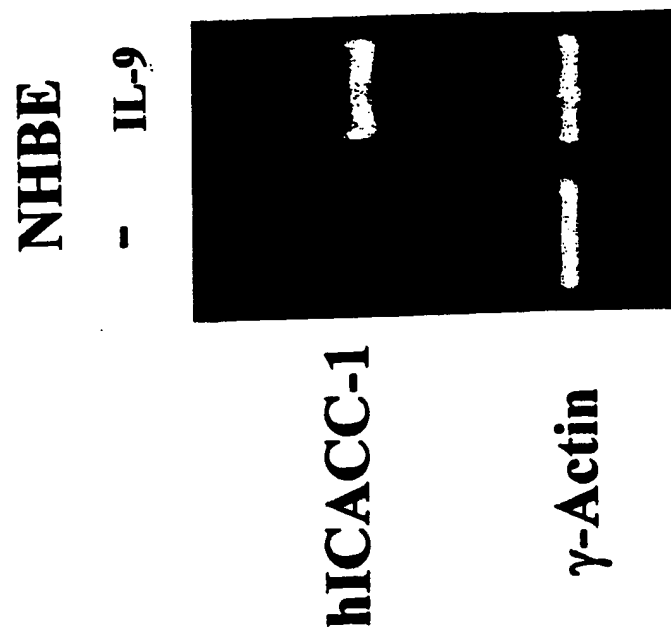


Fig 13

**Fig 14**

Validation of IL-9 Inducibility of Human ICACC-1

Human ICACC-1 is induced by IL-9 in human primary lung cultures

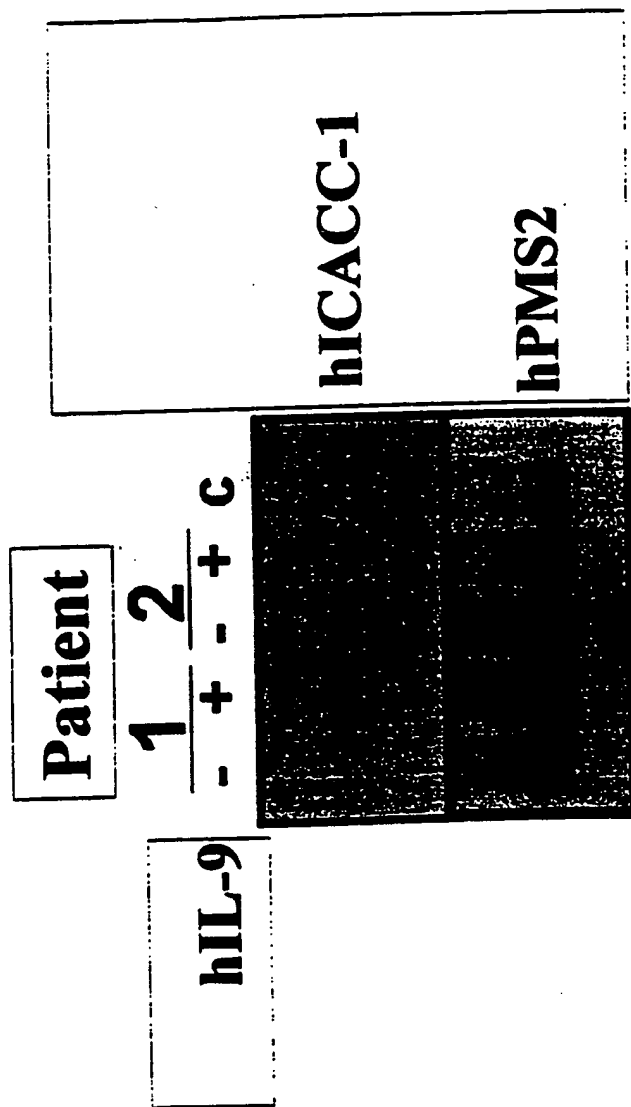


Fig 15

Immunoprecipitation with ICACC-1 Antiserum

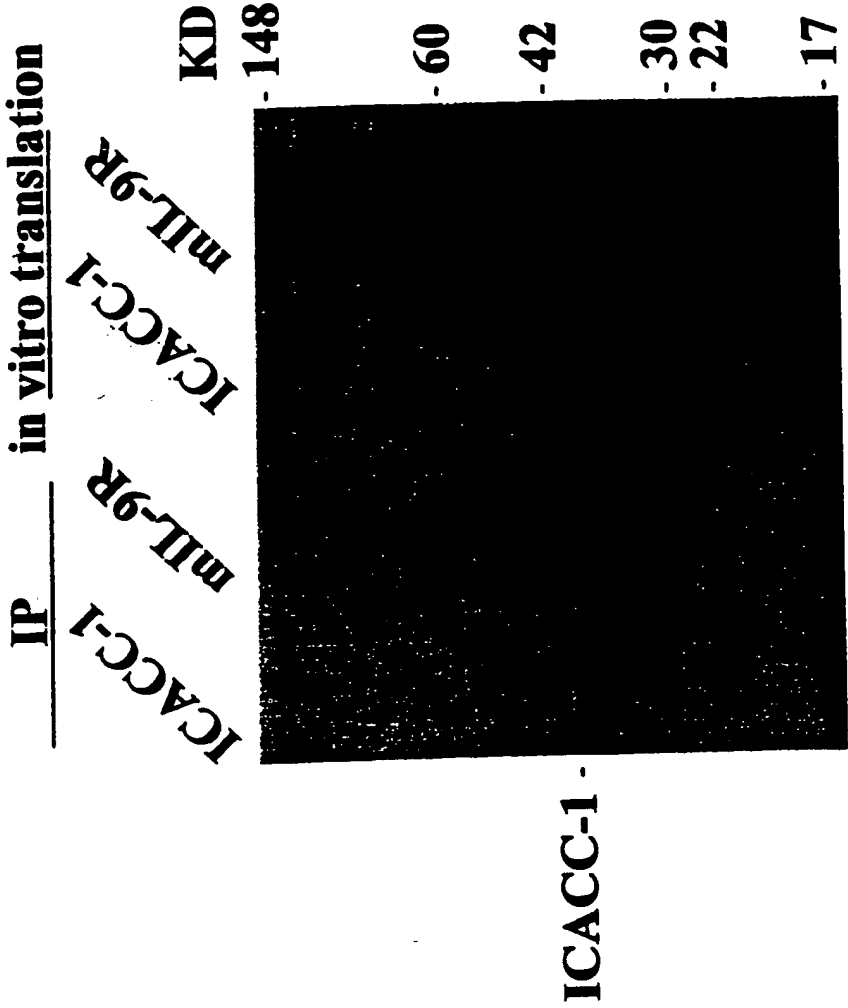


Fig 16

IL-9 Induces Chemokine Production *ex vivo*

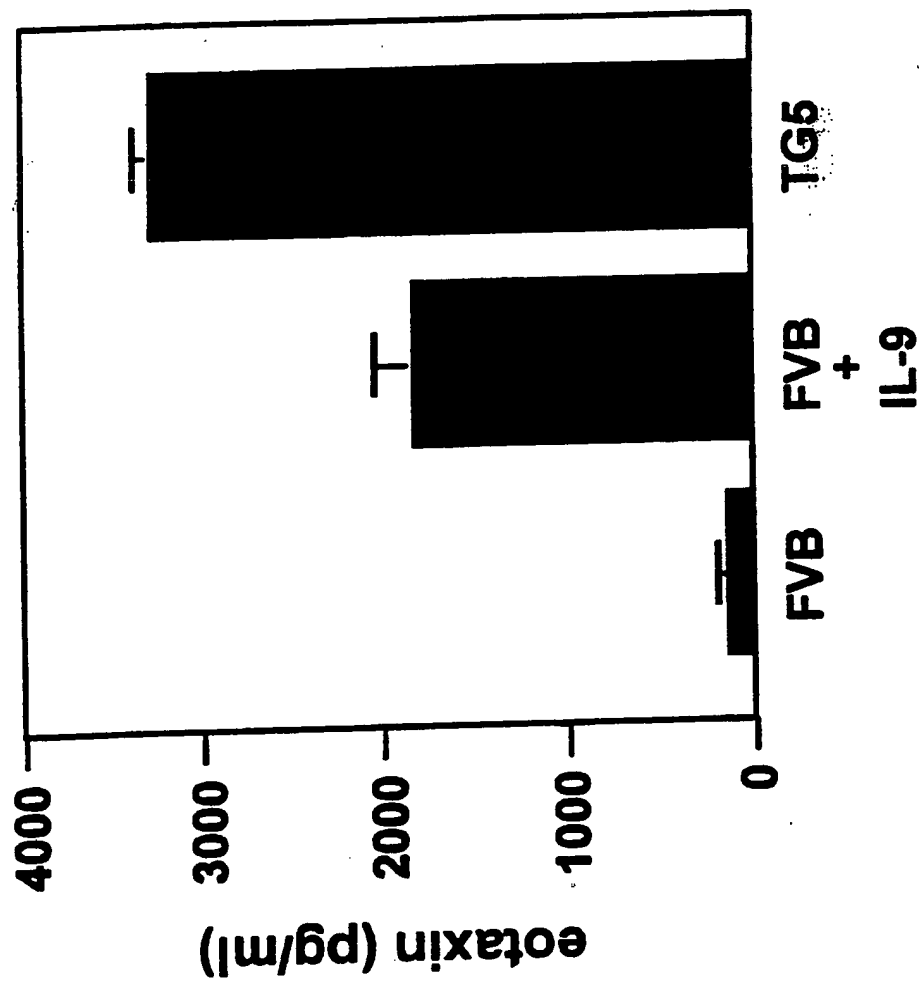


Fig 17

Percent inhibition of IL-9 induced eotaxin in lung culutres by chloride channel inhibitors

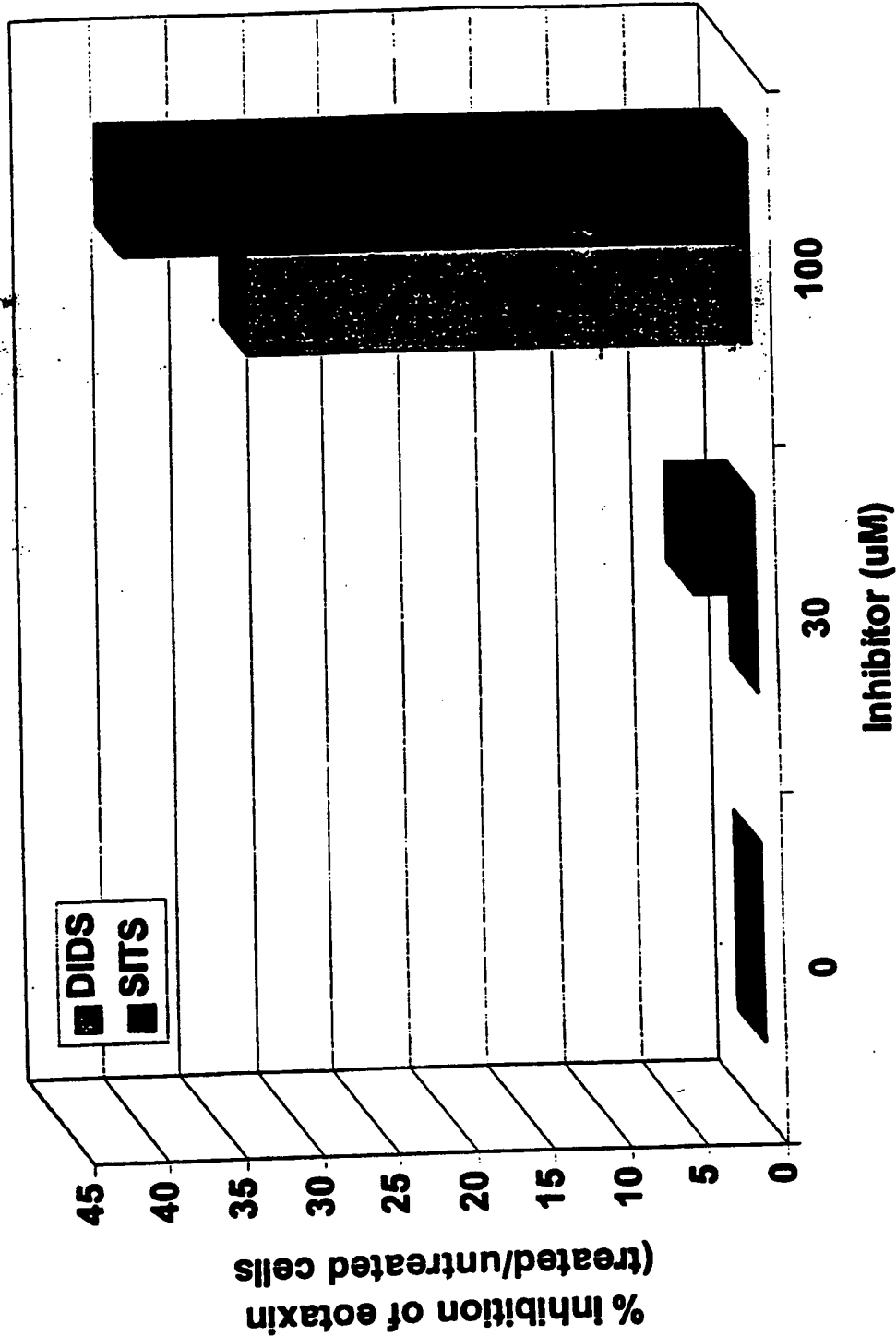


Fig 18

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/04703

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/70, 39/395; C07H 21/04; G01N 33/53

US CL : 424/130.1; 435/7.1; 514/44; 536/23.5

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1; 435/7.1; 514/44; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS DIALOG, MEDLINE, EMBASE, BIOSIS, LIFESCI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96/39419 A1 (YU ET AL) 12 DECEMBER 1996(12-12-96), see entire document.	1-13, 25, 30 and 31



Further documents are listed in the continuation of Box C.



See patent family annex.

*

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L

document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O

document referring to an oral disclosure, use, exhibition or other means

P

document published prior to the international filing date but later than the priority date claimed

T

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Z

document member of the same patent family

Date of the actual completion of the international search

06 JUNE 1999

Date of mailing of the international search report

09 JUL 1999

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